DEPARTMENT OF FOOD AND AGRICULTURE

EXECUTIVE SUMMARY
Of Report BC 90-1 Entitled
"Monitoring of the Spring, 1987 Field Release of Genetically Engineered Bacteria in Contra Costa County"

Environmental Monitoring and Pest Management Branch
Division of Pest Management, Environmental
Protection and Worker Safety
Department of Food and Agriculture

PURPOSE:

Frost injury in strawberry plants is catalyzed by ice-nucleating bacteria which occur naturally on the leaves. This ability to form a nucleus for ice formation is due to a particular structural feature of the bacterium's surface. Other naturally occurring strains of the same species of bacteria do not have this structural feature and do not serve as nuclei for ice formation.

Bacteria which are similar to the naturally occurring non-nucleating, or "ice minus" bacteria were genetically engineered and applied to strawberry plants in hopes that they would competitively displace the ice-nucleating strain and thereby protect the strawberry plants from frost injury.

Since this is one of the first genetically engineered products to reach the field testing stage, there was a great deal of interest in the persistence and off-site movement of ice minus bacteria after application. The objectives of this study were:

1. To determine whether genetically engineered ice minus bacteria could be detected over time on vegetation in a strawberry field in Contra Costa County.

2. To determine whether they could be detected in air or on vegetation outside the release site.

BACKGROUND:

Since the ice-nucleating bacteria which normally occur on strawberry leaves are responsible for frost damage of the plant, they are considered pests by state and federal law, and as a result the ice minus strain, which will be applied to reduce the numbers of the ice-nucleating variety, is classified as a pesticide (see the Federal Insecticide, Fungicide and Rodenticide Act, Section 3, and the California Food and Agricultural Code Sections 11404 and 12753).

Both state and federal law require that pesticides be registered before they can be sold for use. In preparation for registration, the manufacturer tests the pesticide to determine the effect it will have on human health, wildlife and the environment, and then submits this information to federal and state officials for evaluation. The field trials reported here were conducted by the manufacturer, Advanced Genetic Sciences (AGS), as a part of that registration process.

AGS obtained permission from state and federal authorities to test their experimental product. As a condition of that permission, called a research authorization, and as a result of the high degree of interest generated by one of the first releases of a genetically engineered product in California, the Department of Food and Agriculture required that the release be monitored by the Environmental Monitoring and Pest Management Branch. This report is the result of that monitoring.

STUDY METHODS:

The study area, consisting of a single strawberry field less than 1/4 acre in size, was located south of Brentwood in Contra Costa County. On 24 April and 12 May, the ice minus bacteria were applied to strawberry plants using a hand-held garden sprayer. To reduce drift, applications were made when the wind speed was low. Sixteen air samples were taken around the perimeter of the field by drawing a large volume of air through a collection fluid. Air samples were taken prior to each spray application, the day of the application, and one day after.

Plant samples were taken from the application area and from three off-site locations before and after application, and analyzed for the presence of ice minus bacteria.

MAJOR FINDINGS:

Although genetically engineered bacteria were consistently recovered in air samples collected 15 meters from the study area in all directions, they were almost completely absent from off-site vegetation samples collected up to 4 weeks post-spray. The abundance of genetically engineered and naturally occurring bacteria on strawberry leaflets in the treatment area declined over time to non-detectable levels. This decline most likely resulted from warm, dry weather conditions during the monitoring period which are generally unfavorable for bacterial growth.

CONCLUSIONS:

Although ice minus bacteria were dispersed off-site by air, they did not colonize plants. Either the concentration of bacteria in air was low enough that impaction of perimeter plants was a rare event, or unfavorable weather conditions made colonization of the plants unlikely.

The results of this study indicate that further testing of ice-nucleating bacteria on larger acreages would be warranted.

Ronald J. Oshima
Branch Chief

MONITORING OF THE SPRING, 1987 FIELD RELEASE OF GENETICALLY ENGINEERED BACTERIA IN CONTRA COSTA COUNTY

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ABSTRACT

A monitoring study was conducted by the California Department of Food and Agriculture, Biological Control Program, to determine the on-site persistence, off-site movement in air, and colonization of vegetation by Frostban®, a genetically engineered microbial pesticide, applied in April and May, 1987 in Contra Costa County. Although genetically engineered bacteria were consistently recovered in air samples collected 15 m from the treatment plots in all four cardinal directions, they were virtually absent from off-site vegetation samples collected for up to 4 weeks post-spray. The abundance of genetically engineered and naturally occurring bacteria on strawberry leaflets in treated plots declined over time to non-detectable levels. This decline most likely resulted from warm, dry weather conditions during the monitoring period which are generally unfavorable for bacterial growth.

ACKNOWLEDGMENTS

Thanks to Advanced Genetic Sciences, Inc. for their cooperation in carrying out the study plan.

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Disclaimer

The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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I. INTRODUCTION

Although microbial pesticides have been developed and registered for commercial use to control insect and plant pests in California, there are presently no registered pesticides which utilize recombinant DNA technology. Genetically engineered products are just beginning a phase of environmental testing. Frostban®, which is made up of strains of genetically engineered Pseudomonas species, is one of the first of these new products to reach the field testing stage.

When applied to crops, these genetically engineered Pseudomonas strains are intended to provide an alternative form of frost control through competitive displacement of naturally occurring ice-nucleation active (INA^+) epiphytic bacteria (Lindow, 1982). INA^+ bacteria have been shown to be important catalysts of frost injury in plants (Lindow et. al., 1984). Altered bacteria which lack the ability to initiate frost formation are referred to as INA^- or "ice minus" bacteria.

Final approval of a research authorization was given by the California Department of Food and Agriculture (CDFA), and an experimental use permit by the Environmental Protection Agency (EPA), to Advanced Genetic Sciences (AGS) for the environmental release and testing of Frostban® bacteria on strawberry plants in April, 1987. These pesticide applications constituted the first deliberate release of a genetically engineered microbial pesticide for field testing in the United States.

The CDFA, Biological Control Program (BCP), a program of the Environmental Monitoring and Pest Management Branch, acting in support of this registration action, initiated a monitoring study of the environmental fate of the genetically engineered microbial pesticide applied in spring, 1987.

The objectives of this study were:

1. To determine whether genetically engineered Pseudomonas species could be detected over time on vegetation in a strawberry field specified as the release site for these products in Contra Costa County.

2. To determine whether genetically engineered Pseudomonas species could be detected in air or on vegetation outside of the environmental release site in order to assess whether there had been off-site movement of these products.

The results of this environmental monitoring study are the subject of this report.

II. MATERIALS AND METHODS

Study Area

The study area was located in an agricultural region of Contra Costa County, just south of the city of Brentwood (Figure 1). This location was specified in an experimental use permit approved by the EPA and also in a research authorization given by the CDFA, as the site for release of genetically engineered bacteria in the genus Pseudomonas, by AGS.

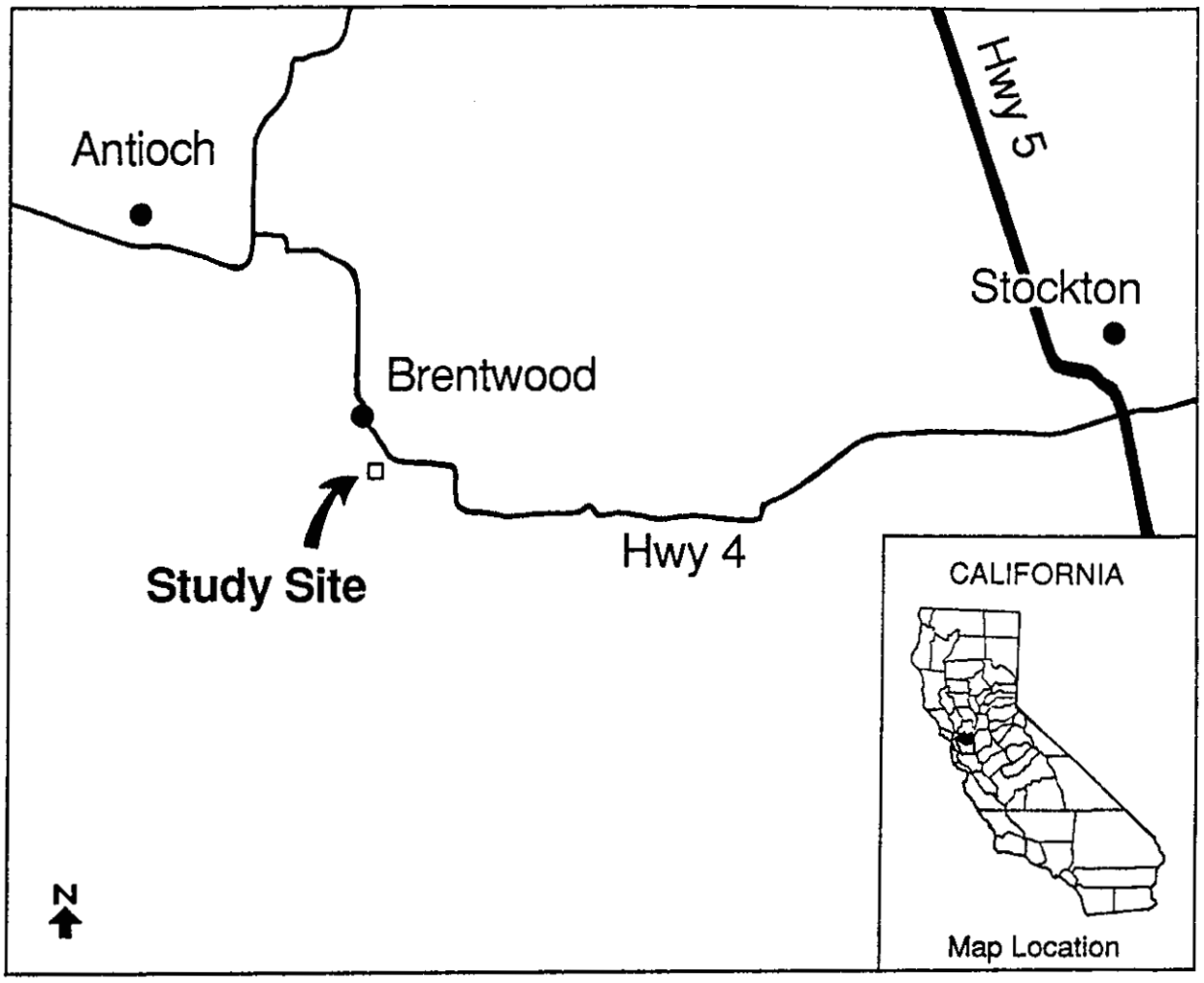
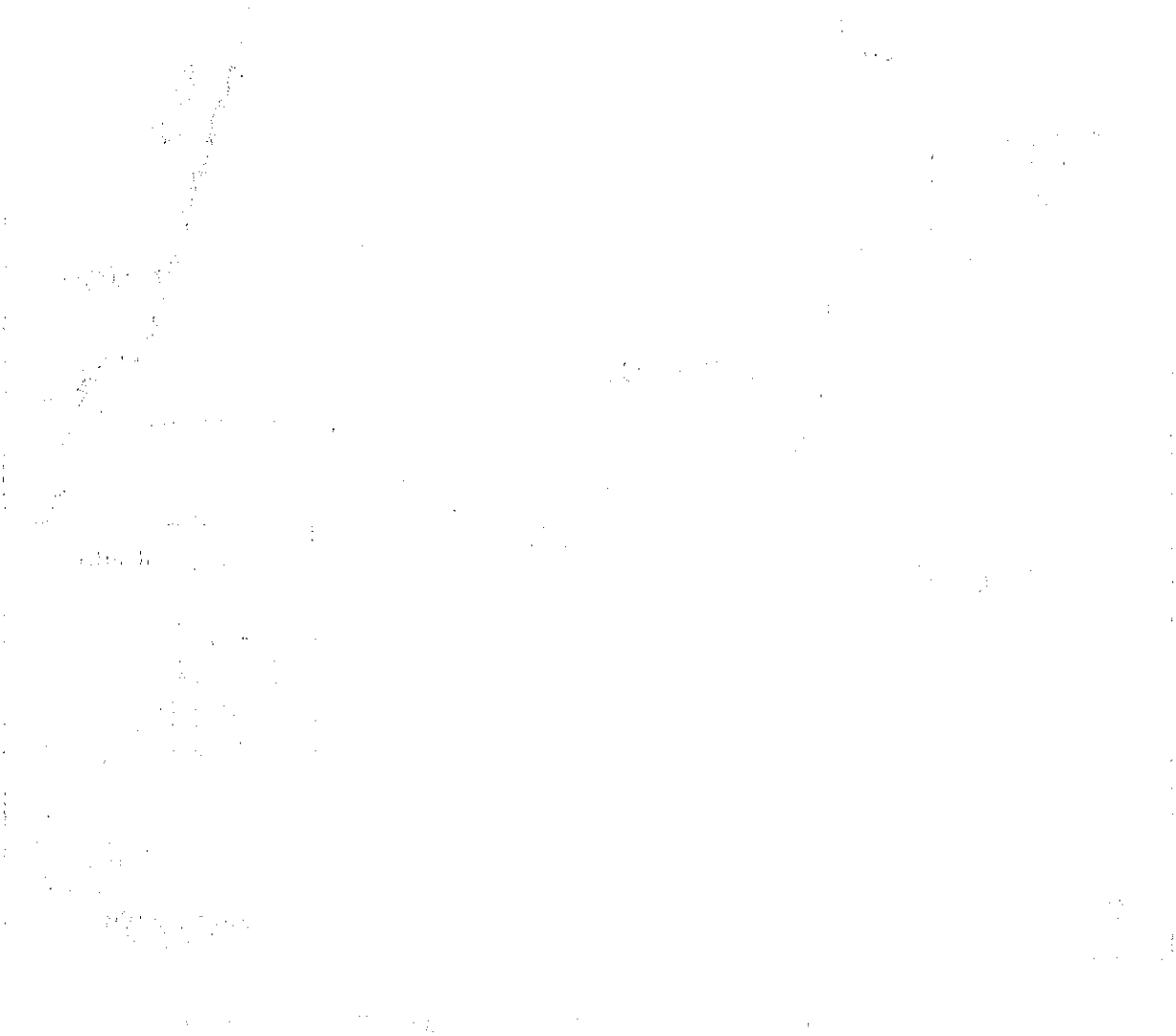


Figure 1. Location of the Frostban® test site in Contra Costa County, California



The experimental site consisted of a single 28.5 m X 28.5 m (0.2 acre) strawberry field divided into 16 equal plots. A bare soil buffer zone 15 m wide surrounded the field (Figure 2). A six foot chain-link fence surrounded the study area. This fence, which was placed 5 m outside the buffer zone on the north and west sides, 10 m outside the buffer zone to the east and 3.3 m outside the buffer zone to the south, was used as a barrier to unauthorized access to the study area.

West and adjacent to the study site was a corn field 20 m from the edge of the experimental strawberry site (Figure 3). A 'top-worked' pear orchard was located 28 m east of the experimental site beyond a dirt access road, and to the north was a second, regular, pear orchard about 60 m from the experimental strawberry site. To the south, 0.7 m from the study site, was a second plot of strawberries the same size as the experimental plot, and across Concord Avenue, approximately 60 m from the study site, was a tomato field. Approximately 0.32 km west of the site were some abandoned buildings with several apricot trees growing around their perimeters. A large mound of soil adjacent to a sump area, and a narrow drainage trough were located to the northeast of the experimental site.

AGS Experimental Design

Since the sole objective of the CDFA study was to monitor the environmental fate of Frostban®, the AGS study design is presented strictly for informational purposes.

Bacterial Strains

Two strains of genetically altered fluorescent bacteria in the genus Pseudomonas were used by AGS for these experiments: Pseudomonas syringae, strain RGP36R2;

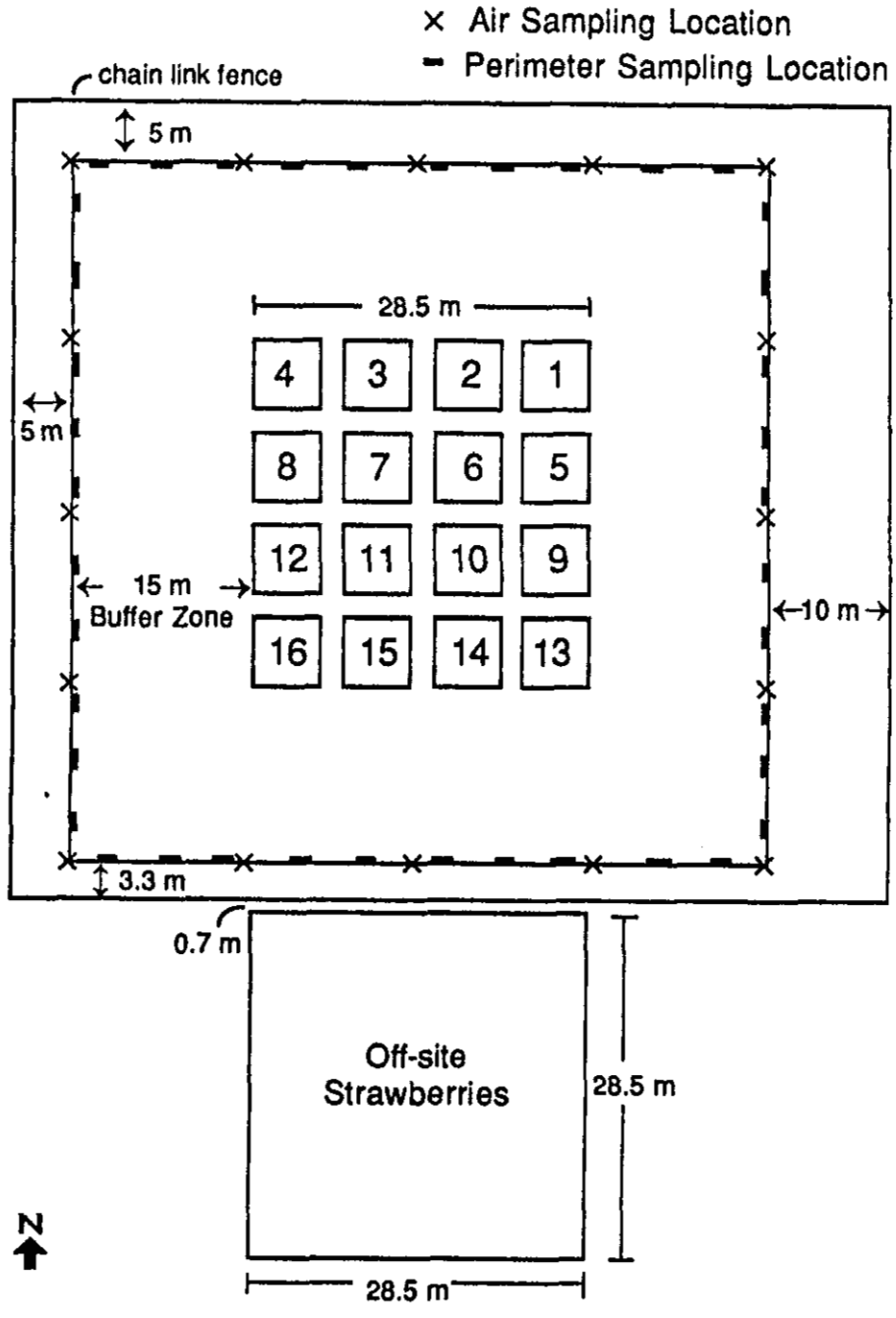


Figure 2. Experimental Site and Vicinity

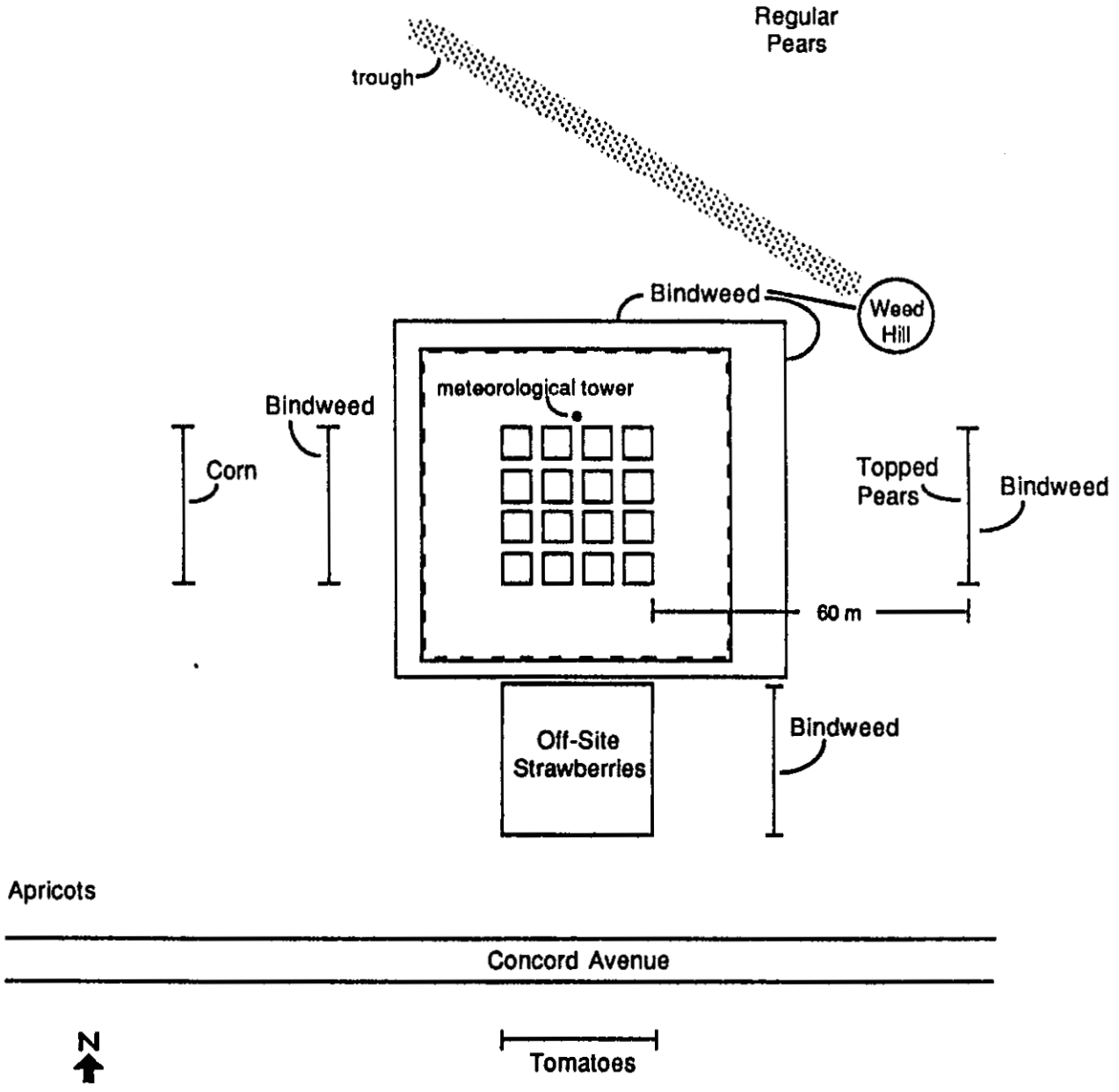


Figure 3. Vegetation Sampling Locations

and Pseudomonas fluorescens, strain GJP17BR2. Both strains were selected for resistance to the antibiotic rifampicin. The use of the trade name Frostban® in this report refers to either or both of these strains. These strains were genetically modified by deleting a sequence of 400 base pairs from the gene which codes for an ice nucleation active protein on the bacterial surface. This deletion renders the bacteria relatively inactive as ice nuclei.

Study Design: The experimental design was a 4 X 4 Latin Square (i.e., 4 treatments replicated 4 times). Each of the 16 plots contained 7 beds of strawberry plants (0.8 m centers), measured 5.97 m on a side and was separated from adjacent plots by 1.7 m (i.e., 2 beds). The treatments were applications of: genetically altered Pseudomonas syringae (in buffer); genetically altered Pseudomonas fluorescens (in buffer); buffer alone (control); and a combination of a fungicide and a bactericide. Each treatment was applied to four plots on each of two treatment dates. Applications were made on 24 April, 1987 and again on 12 May, 1987. The initial application was to have taken place when approximately 10% of the plants were in bloom, and the follow-up when 80% bore flowers. However, on the morning of the first spray, vandals uprooted most of the plants, destroying the flowers in the process, and delaying subsequent bloom.

The purpose of the AGS study was to quantitate survival, dispersal, and competitiveness of Frostban®, and to evaluate the potential of these strains to reduce local populations of INA+ bacteria and subsequently limit frost damage in treated plants, thereby verifying experimental laboratory data.

Dosage: Each treatment of genetically engineered bacteria contained approximately 2 X 10^8 cells per ml in a volume of 10 liters of water.

Treatment Equipment: Applications of Pseudomonas species and other treatments were made using a Hudson hand-pump low pressure hand-held garden sprayer. To reduce drift, the spray nozzle was held close to the ground (within 0.5 m of the plant canopy). To further mitigate drift, applications were only made when wind speeds at the canopy level were below 2.24 m/s. Due to higher wind speeds on the second date of application, there was a 96 minute delay between the two bacterial sprays.

CDFA Experimental Design

Air Sampling - Air samples were taken with low volume, high velocity, all glass impingers (AGIs), which were custom-designed and manufactured (Figure 4). Air flow through these samplers was 40-50 L/min. The collection fluid was a 0.01 M phosphate buffer solution. Each impinger was supplied with 50 ml of this fluid so that the distance between the tip of the capillary tube and the top surface of the collection fluid was 2 mm.

Sixteen AGIs were placed around the perimeter of the sampling area, four on each side, at the outlying edge of the buffer zone (Figure 2). All samples were collected at a height of exactly one meter above the ground.

Air samples were taken eight days prior to the first spray application, the day of application, and one day post-spray. Air samples were also taken one day before the second spray, the day of the second application, and one day post-spray. All 16 AGIs were used for each of these sampling days. All samplers

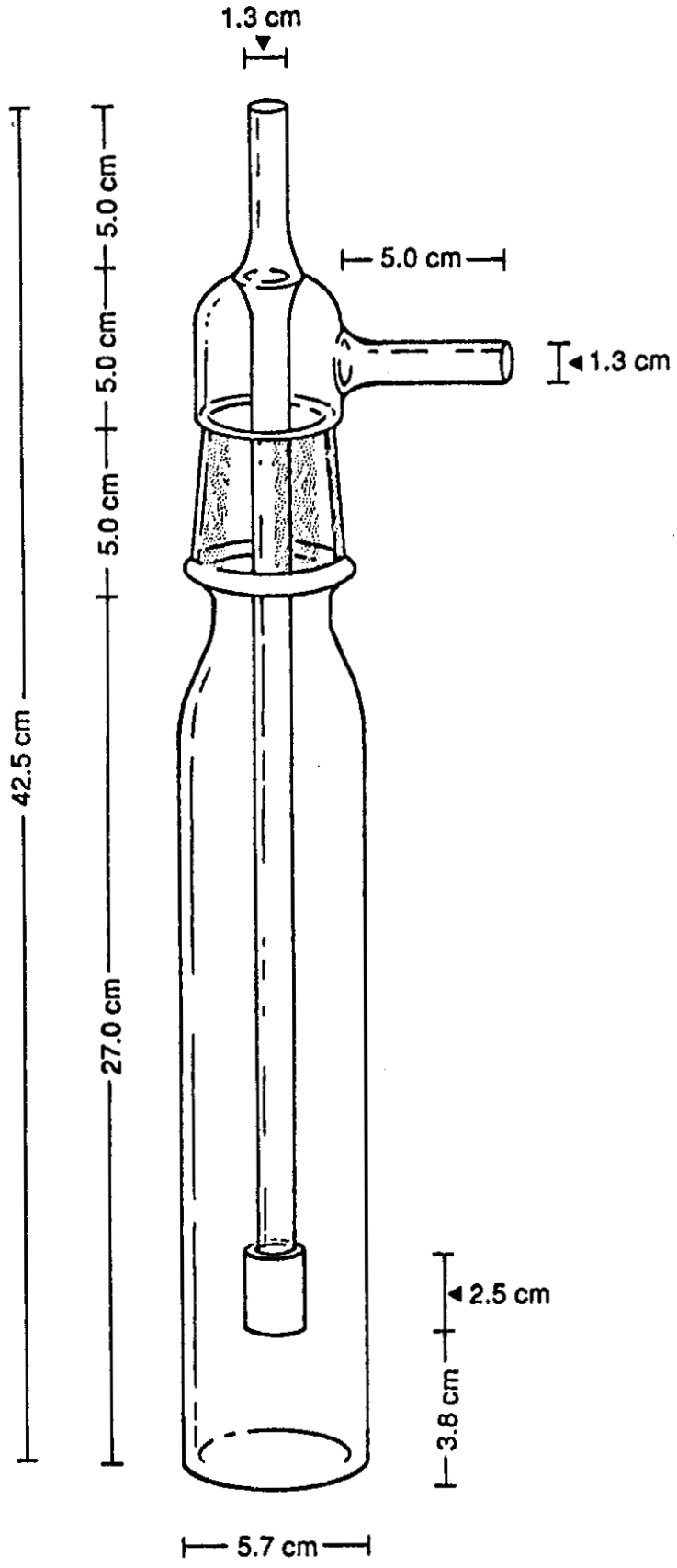


Figure 4. Diagram of all glass impinger.

were run continuously for 30 minutes except the day of the first spray application when samplers were run for 36 minutes, and the day of the second spray when samplers were run for 15 minutes, shut-off for 81 minutes during a period of excessive wind speeds, and then run for an additional 15 minutes when the wind speed decreased to below 2.24 m/s.

On-Site Vegetation Sampling - The CDFA monitoring protocol called for sampling all 16 treatment plots (Figure 2). For each sampling date, one sample of 25 randomly collected strawberry leaflets (one per plant), was collected from each plot. These samples were taken on the day of the first spray and 7, 14, 21, 28, and 35 days post-spray. In addition, on the day of the first spray, one sample of 25 randomly collected strawberry blossoms (one per plant), was also collected from each plot. However, the study site was vandalized prior to the spray event and further flower production was delayed.

Off-Site Vegetation Sampling - Additional vegetation samples were taken from three off-site sources: (1) from oat plants grown in flats, (2) from strawberry plants in a field adjacent to the study site, and (3) from various plant species in orchard and row crop locations surrounding the study site.

Background samples were taken 14 days prior to the first spray. Additional vegetation samples were taken on the day of, and 7 days after the first Frostban® application, and 1, 7, 13, 21, and 28 days after the second spray. Each sample consisted of 25 leaflets, or 25 blossoms.

Oat plants grown in forty 25.4 cm x 50.8 cm flats were placed at even intervals around the perimeter of the buffer zone. Each flat contained approximately 200 oat seedlings that were 2-2 1/2 weeks old and were approximately 15.2 cm tall

(on the day of the first spray). Each biological screen sample collected from oat plants was comprised of 25 randomly chosen leaves. Samples were taken on the day of the first application, and 1 and 7 days post-spray. For the second spray event, samples were collected 1 and 7 days post-spray.

Four meters south of the buffer zone was a second strawberry field measuring 28.5 m on a side. On each sampling date, a total of 10 samples was taken from this field. Initially, eight leaf samples and two blossom samples were taken. As the numbers of flowers diminished over the course of the study, the blossom samples were replaced with leaf samples. Each leaf sample was comprised of 25 randomly selected strawberry leaflets (one per plant), and each blossom sample contained 25 randomly picked flowers (one per plant).

Field bindweed (Convolvulus arvensis L.), was collected at several locations in and around the study site. Four bindweed samples were taken on each sampling date with the following exceptions: on the day of the first spray, three samples were taken, and 28 days after the second spray, five samples were collected. Two corn leaf samples were taken on each sampling date. One sample of apricot leaves was taken on each sampling date from a series of trees located around old buildings near the study site. Three leaf samples were taken on each sampling date from regular pear trees in rows adjacent to the study site. Nine leaf samples were taken on each sampling date from top-worked pear trees in rows adjacent to the study site, except for the day of the first spray when five pear leaf samples were taken and four mixed weed samples were taken from between the tree rows. One sample of tomato leaves was taken on each sampling date from a field south of the study site across Concord Avenue. Mixed weed species were collected from the perimeter of a mound of soil at the northeast corner of the study area, 14 days prior to the first spray and on the day of the first spray.

In addition, various weed species were collected 28 days after the second spray on the strawberry study plot after the plot had been disced.

All vegetation samples were stored in coolers until they could be transported to Sacramento where they were refrigerated at 4°C in locked rooms until delivered to the CDFA Analysis & Identification (A&I) laboratory.

Quality Control

For each week of sampling, including background samples, six quality control samples were created and interspersed with field collected samples prior to transport to the A&I laboratory. Strawberry leaflets similar to those at the study site were inoculated with one or both species of genetically engineered bacteria.

Weather Monitoring

Meteorological data collected by EPA using equipment located just to the north of the study plots (Figure 3), before, during, and one day after each pesticide application included: minimum, maximum and mean wind speed ($m\text{-}sec^{-1}$); minimum, maximum, and mean temperature in degrees Celsius; percent relative humidity, direct and reflected solar radiation ($W\text{-}m^{-2}$); vertical wind speed ($m\text{-}sec^{-1}$); and the percent of the time the vertical wind direction readings indicated upward, downward or no movement (Appendix V).

Information on air temperature was collected at canopy level, 1m, 2m, 3m, and 10m heights; relative humidity and vertical wind speed at 1m, and solar radiation at a height of 1.5m. Collection of weather information occurred during the 30 minute sampling period for each air sampling event.

Additional weather information was collected from 15 April, 1987 to 15 June, 1987 in Brentwood, California, by the California Department of Water Resources (Appendix V).

Laboratory Procedures

Preparation of Samples - All plant material from each sample was transferred to sterile half-gallon vessels containing 10 ml of sterile distilled water per gram of tissue, sealed, and placed on a reciprocating shaker at 250 rpm for 2 hours. Unconcentrated wash buffer from on-site samples was used to inoculate selective media containing rifampicin and/or cycloheximide (Appendix I). A 50 ml aliquot of wash buffer from samples collected off-site was centrifuged at 7500 rpm for 10 minutes to concentrate the bacteria. Excess wash water was decanted, the pellet resuspended in the remaining 5 ml, and the concentrated suspension used to inoculate selective media containing rifampicin and/or cycloheximide. For each culture plate, 0.1 ml of wash water was uniformly spread to inoculate the surface of selective media. Plates were then sealed with parafilm, inverted and incubated on a laboratory bench at room temperature.

Evaluation of Samples - After 48 to 72 hours at room temperature, culture plates were rated for growth of Frostban® bacteria according to the number of individual colonies on the selective media as follows: (0) indicated no colonies; (+) indicated 1 to 10 colonies; (++) 11 to 100 colonies; (+++) more than 100 colonies; and O.G. indicated that the plate was overgrown. Thus, there were 5 levels of bacterial abundance which will subsequently be referred to as levels 0-4.

Using the sample preparation procedure summarized above, each individual colony on selective media represented 10 colony forming units (CFU) per milliliter of

unconcentrated wash water (10^2 CFU per gram of leaf tissue). Following concentration, each individual colony on selective media was equivalent to one CFU per milliliter of unconcentrated wash water (10 CFU per gram of leaf tissue).

All plates with bacterial growth were illuminated under ultraviolet light to detect the presence of any Pseudomonas type bacteria similar to the Frostban® organisms. Distinct fluorescent colonies were identified, circled, examined for morphological type and transferred to plates of King's B medium (see Appendix I) for further characterization. A maximum of five colonies of each morphological type were characterized in this way for each sample analyzed.

Distinct colony morphology characteristics were: for genetically engineered P. syringae, a medium colony which developed a convoluted edge and became crater-like as it developed a distinct cloudy white cast; and for genetically engineered P. fluorescens, a small colony usually with a smooth edge which sometimes became domed and viscous. Only isolates which were resistant to rifampicin, fluorescent, and had correct colony morphology, were tested further.

After 48 hours, isolates on King's B medium were tested for the oxidase and arginine reactions and ice nucleation activities (Appendices II-IV). Only isolates which had the proper profile for arginine dihydrolase and oxidase tests (Appendix I) were sent for gene probe analysis. Bacteria from field samples and laboratory quality control samples were transferred to Luria broth prior to delivery to Dr. Bostock at U.C. Davis (UCD) for gene probe analysis (Appendix I). The gene probe technique involved extraction of DNA from test isolates, restriction enzyme digest using two enzymes, Eco RI and PvuII, electrophoresis on an agrose gel, followed by transfer of DNA onto a Nytran membrane (Schleicher

and Schull) for southern blot analyses. Two probes were prepared with [^{32}P] labelled DNA, one utilizing the INA Z gene of P. syringae and the other the INA W gene of P. fluorescens. Blots were incubated at 43°C in the presence of probes for 8 hrs and autoradiograms were prepared.

Restriction patterns from test isolates were compared with those standards to evaluate identity. Naturally occurring bacteria and the two species of test bacteria isolated from the Brentwood release site could be distinguished on the basis of unique restriction patterns for each test isolate of genetically engineered bacteria.

A positive determination of genetically engineered P. syringae was made if the following criteria were met: growth on rifampicin, fluorescense, oxidase negative, arginine dihydrolase negative, negative for ice nucleation,and positive identification as genetically engineered P. syringae through gene probe analysis. A positive determination of genetically engineered P. fluorescens was made if the following criteria were met: growth on rifampicin, fluorescence, oxidase positive, arginine dihydrolase positive, negative for ice nucleation activity, and positive identification as genetically engineered P. fluorescens through gene probe analysis. Isolates that failed to meet the criteria for either genetically engineered P. syringae or P. fluorescens were considered to be naturally occurring Pseudomonads.

Quality Control Samples - Isolates of genetically engineered P. syringae and P. fluorescens used as controls for this procedure were grown on King's B medium for 36 to 48 h at ambient laboratory temperature (21.1°C - 23.9°C). Individual colonies were then selected and used to inoculate 50 ml of nutrient broth

amended with 50 mg/l rifampicin, contained in 250 ml Erlenmeyer flasks wrapped with aluminum foil. Flasks were placed on a shaker and rotated at 200 rpm for 36 to 48 h at ambient laboratory temperatures.

A 10 ul aliquot from the 36 to 48 h nutrient broth culture was transferred to a flask containing 100 ml of sterile peptone buffer forming a bacterial suspension containing one of the genetically engineered Pseudomonas species. After mixing for 15 seconds with a vortex mixer, this diluted bacterial suspension was transferred to a sterile Chromist® jar. Each quality control sample was composed of 25 strawberry leaflets collected from strawberry plants grown outdoors in Sacramento. Using a Chromist® sprayer, both the upper and lower surfaces of the leaflets were sprayed with inoculum to runoff.

Statistical Analysis

To determine if there was an effect of time or treatment in the probability of recovering bacteria on each media type from plots treated with either genetically engineered P. syringae or P. fluorescens, logistic regression analysis was performed using the methods of Feinberg (1977) and the CATMOD procedure (SAS, 1986). Abundance values of 0 and 4 were never observed for media amended with cycloheximide only and cycloheximide with rifampicin, respectively. Because of the lack of observations or low number of observations for treatments for each date, levels (1,2) and (3,4) were collapsed to produce a 2 x 7 table of bacterial abundance versus time for media amended with cycloheximide only. Levels (0,1) and (2,3) were collapsed to produce a 2 x 7 table of abundance of bacteria on media amended with both cycloheximide and rifampicin. This was justified because of the ordered nature of the abundance categories. The resulting two response levels were treated as ordered categories in logistic analyses with time considered as continuous, and treatment a class variable.

Ordered categorical analyses using the method of maximum likelihood to determine expected cell counts were carried out using the approach of Feinberg (1977). Only response levels which were observed for both media types (levels 1, 2, and 3) were included in these analyses.

III. RESULTS

Weather Monitoring

The mean wind speeds measured for the 30 minute air sampling period during background air sampling, during and after the first spray event were 1.0, 0.1 and 1.4 m/s, respectively, at 1 m (Appendix V). The mean wind speeds for the 30 minute air sampling period after the second spray event were 1.3, 0.6 and 2.6 m/s, respectively. Wind direction during the first Frostban® application was from the northeast, east, and south, while during the second spray event wind direction was from the south, southwest, west and northwest (Figure 5). The relative humidities during the first and second spray events were 65% and 47%, respectively. Mean temperature at canopy level was 12.7°C during the first spray period, and 24.5°C during the second spray period.

Measurable amounts of precipitation were detected on 29 April, 30 April, and 20 May, 1987. During the monitoring period, there were several periods of unseasonably warm weather. Average temperatures were over 21.1°C during the weeks ending 9 and 16 May, 6 and 13 June, 1987 with maximum temperatures during those weeks reaching 33.3, 32.2, 31.7, and 32.8°C, respectively (Appendix V). Relative humidities were correspondingly depressed during those same periods.

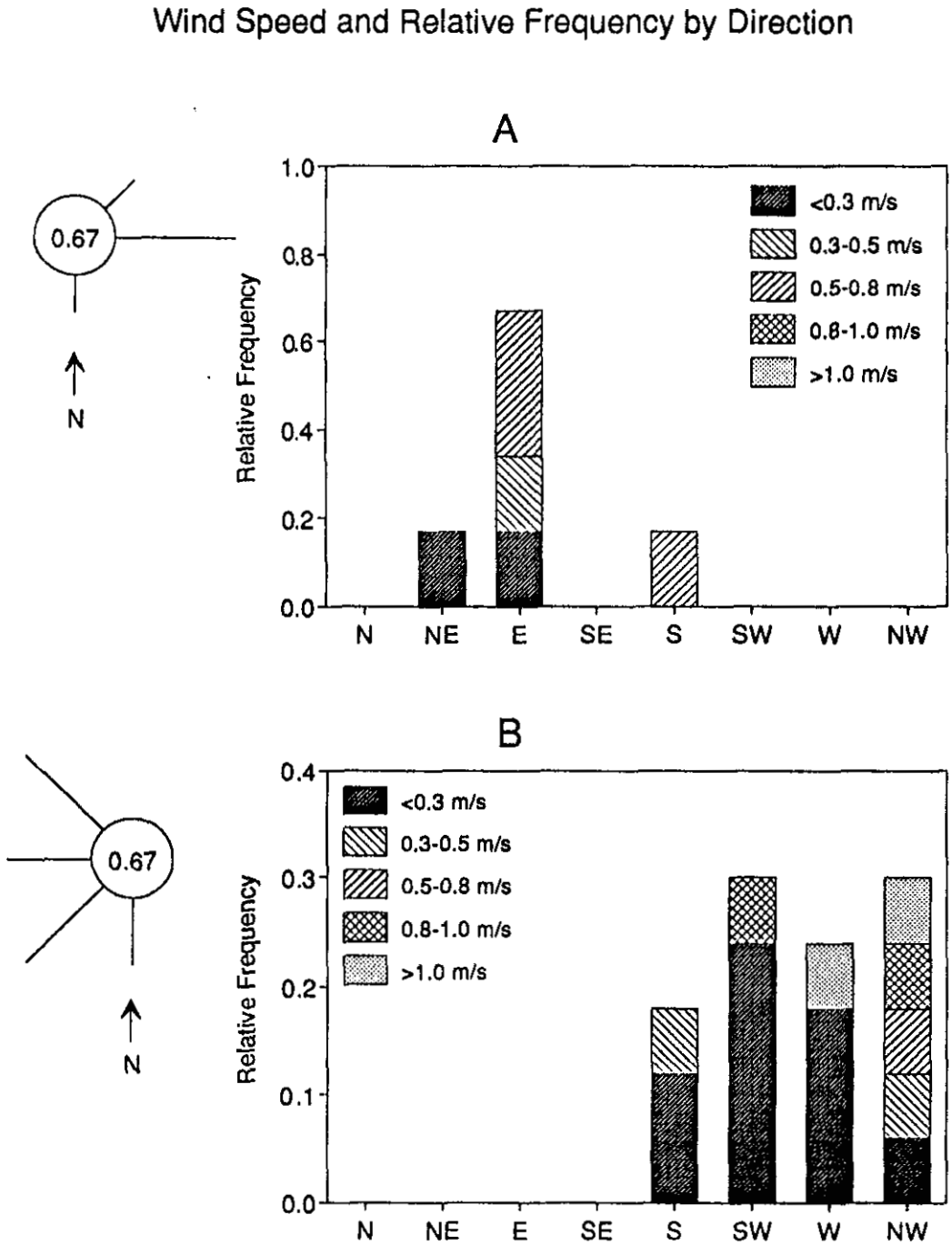


Figure 5. Windrose data and histogram of wind speed and direction at one meter height during the first and second Frostban® applications, April 24, 1987 (A), and May 12,1987 (B). (From Seidler and Hern,1988.)

Air Samples

Genetically engineered bacteria were detected in 75% (n= 16) and 56.3% (n=16) of air samples collected during the first and second Frostban® applications, respectively (Figures 6 and 7). During the first application, genetically engineered P. fluorescens was detected in 66.7% (n= 12) and P. syringae in 33.3% (n= 12) of positive air samples. Genetically engineered P. fluorescens was identified in 55.6% (n= 9) and P. syringae in 44.4% (n= 9) of the positive air samples collected during the second Frostban® application.

Concentrations of Frostban® bacteria at locations where there were positive recoveries during the first application were within a range of 0.57 to 6.9 CFU/m^3 air (Table 1). While Frostban® bacteria were recovered from a greater number of air samples during the first spray, a greater concentration of genetically engineered bacteria were recovered from air samples during the second spray. Concentrations of Frostban® bacteria during the second application at sampling locations where there were positive recoveries were within a range of 0.62 to 654 CFU/m^3 air.

No genetically engineered bacteria were recovered from air collected at southeastern sampling locations during either Frostban® application. Frostban® bacteria were not detected in air sampled at one northern and three northeastern locations during the first and second applications, respectively. No genetically engineered bacteria were detected in air samples collected before (background) or one day after each Frostban® application.

Vegetation Samples

On-Site Samples - No genetically engineered bacteria were recovered from foliage

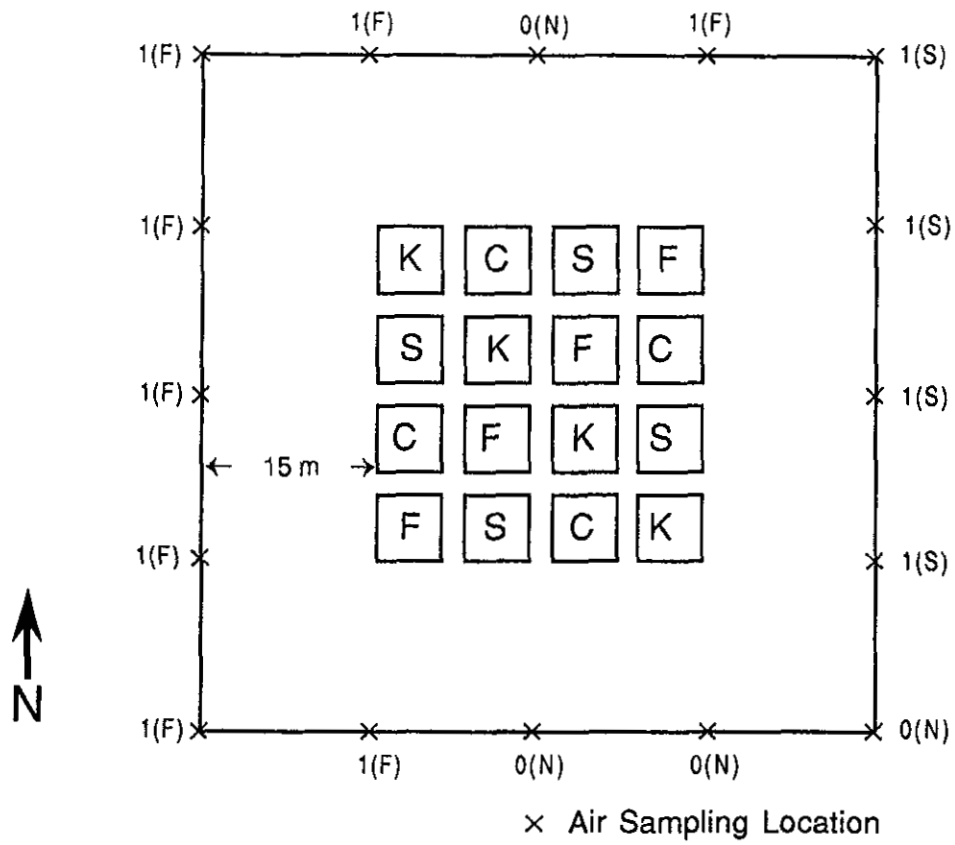


Figure 6. Plot design and abundance index of rifampicin resistant fluorescent bacteria recovered from AGI's during the first spray event, April 24, 1987. Treatments applied to strawberry plots included: F = genetically engineered P. fluorescens; S = genetically engineered P. syringae; C = control buffer only; K = bactericide only. Numbers indicating amount of bacteria recovered in AGI's (0 = none, 1 = 1 to 10 cfu/AGI) are followed by letter in parenthesis indicating bacterial strain isolated (F and S as above, N = no genetically engineered bacteria detected).

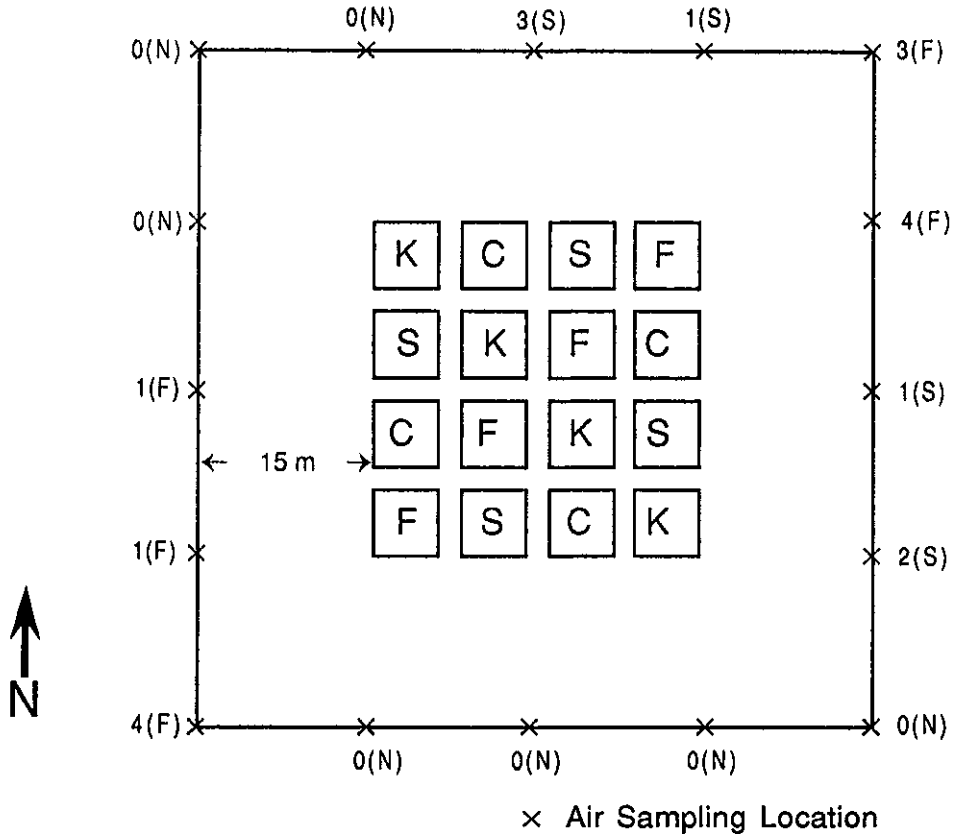


Figure 7. Plot design and abundance index of rifampicin resistant fluorescent bacteria recovered from AGI's during the second spray event, May 12, 1987. Treatments applied to strawberry plots included: F = genetically engineered P. fluorescens; S = genetically engineered P. syringae; C = control buffer only; K = bactericide only. Numbers indicating amount of bacteria recovered in AGI's are followed by letter in parenthesis indicating bacterial strain isolated (F and S as above, N = no genetically engineered bacteria detected). Abundance categories: 0 = none; 1 = 1 to 10 cfu/AGI; 2 = 11 to 100 cfu/AGI; 3 = 101 to 1000 cfu/AGI; 4 = too numerous to count.

Table 1. Recovery of rifampicin resistant Pseudomonads from air samples during Frostban® applications.

Sampling Location	Application 1 L/Min.	Application 1 Abundance Category[1]	Application 1 CFU/m^3 Air	Application 2 Abundance Category	Application 2 CFU/m^3 Air
1	48	0	0	0	0
2	53	0	0	0	0
3	58	1	.57 to 5.7	0	0
4	48	1	.69 to 6.9	4	TNTC
5	53	1	.63 to 6.3	1	.63 to 6.3
6	48	1	.69 to 6.9	1	.69 to 6.9
7	55	1	.60 to 6.0	0	0
8	52	1	.64 to 6.4	0	0
9	49	1	.68 to 6.8	0	0
10	53	0	0	3	63.5 to 629
11	54	1	.62 to 6.2	1	.62 to 6.2
12	51	1	.65 to 6.5	3	66 to 654
13	53	1	.63 to 6.3	4	TNTC
14	48	1	.69 to 6.9	1	.69 to 6.9
15	55	1	.60 to 6.0	2	6.7 to 60.6
16	53	0	0	0	0

1. Abundance categories: 0= none; 1= 1 to 10 CFU/AGI; 2= 11 to 100 CFU/AGI; 3= 101 to 1000 CFU/AGI; 4= too numerous to count (TNTC).

samples collected before the first Frostban® application (Appendix IV). Throughout the study period, no Frostban® bacteria were recovered from plots treated with either biocide, or buffer only (control). In general, Frostban® bacteria were recovered only from plots where they were applied. However, on 24 April, 1987 genetically engineered P. syringae was recovered from a plot to which only genetically P. fluorescens had been applied. Genetically engineered P. syringae, was recovered from all plots to which it was applied from the day of first Frostban® applications until 26 May, 1987, fourteen days after the second spray, whereas genetically engineered P. fluorescens was recovered less frequently over time from treated plots from 24 April to 13 May, one day after the second Frostban® application. Genetically engineered P. fluorescens was recovered frequently over time from treated plots, with some plots sampled in late May failing to yield any genetically engineered bacteria (Appendix IV). On 2 June, 1987, three weeks after the second Frostban® application, no genetically engineered Pseudomonads could be detected in any strawberry foliage samples collected from on-site plots. No Frostban® bacteria were recovered from weed samples collected from on-site locations on 9 June, 1987, two days after destruction of strawberry plants in the experimental plots.

The second spray of Frostban® bacteria did not, in general, increase the abundance of these bacteria to levels found after the first application or above levels found the week before the second application (Appendix II, Table 4).

Statistical analyses using a logistic regression model with response levels treated as ordered categorical variables indicated that there was a highly significant linear decline over time ($p<.0002$) and a significant difference between treatment with P. syringae or P. fluorescens ($p<0.014$) in the abundance of bacteria detected on culture media amended with both rifampicin and cycloheximide

(Table 2). These rifampicin resistant bacteria, recovered from treated plots, can be considered genetically engineered based on the positive identification of multiple isolates and the consistent absence of rifampicin resistant bacteria from control plots. There was a significantly higher abundance of rifampicin resistant bacteria in plots treated with P. syringae as compared with P. fluorescens. Logistic regression analysis indicated that the treatment x time interaction for the above comparison was not significant ($p<.43$).

Statistical analysis with a logistic regression model indicated that there was a highly significant decline over time but no significant effect of treatment on the abundance of bacteria detected on culture media amended with cycloheximide only (Table 3). The statistical analysis concerning the treatment x time interaction was inconclusive. However, examination of the frequency table of the two treatments showed that they were nearly identical in their pattern of decline over time and a treatment x time interaction was excluded on this basis (Table 4).

Ordered categorical analyses including both media types indicated that there was a significant effect of media on the abundance of bacteria ($p= .0053$) and confirmed the significance of time and treatment on bacterial abundance (Table 5). The two-way interactions between variables in the above comparison were not significant according to ordered categorical analysis, nor was the three-way interaction as determined by a separate analysis.

Off-Site Samples - Genetically engineered P. fluorescens was detected in one out of 464 (0.2%) off-site vegetation samples. This positive sample consisted of oat foliage collected from the edge of the buffer zone in the northwest corner

Table 2. Analysis of variance results for abundance of bacteria on media amended with cycloheximide and rifampicin using a logistic regression model[a].

Source of Variation	Degrees of Freedom	Chi-square	Probability
Intercept	1	13.06	.0003
Time	1	13.81	.0002
Treatment	1	6.08	.0137
Likelihood ratio	11	8.31	.69

[a]Logistic model ln (abundance) = 6.87 - 1.66 (time) - 1.47 (treatment)

Table 3. Analysis of variance results for abundance of bacteria on media amended with cycloheximide using a logistic regression model[a].

Source of Variation	Degrees of Freedom	Chi-square	Probability
Intercept	1	12.47	.0004
Time	1	13.21	.0003
Likelihood ratio	5	4.87	.4313

[a]Logistic model ln (abundance)= 7.36 - 1.9 (time).

Table 4. Frequency of recovery (abundance categories combined) of genetically engineered P. syringae (S) and P. fluorescens (F) from treated strawberry plots amended with rifampicin (Rif) or cycloheximide only (Cyclo) for each of 7 weeks following the first Frostban application. The second application occurred on week 4.

	F							S						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
(1,2) CYCLO	0	1	1	1	4	4	4	0	0	0	2	4	4	4
(3,4)	4	3	3	3	0	0	0	4	4	4	2	0	0	0

	F							S						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
(0,1) RIF	0	0	3	3	3	4	4	0	0	0	1	1	4	4
(2,3)	4	4	1	1	1	0	0	4	4	4	3	3	0	0

Table 5. Analysis of variance results for abundance of bacteria using the method of maximum likelihood.

Source of Variation	Degrees of Freedom	Chi-square	Probability
Intercept	2	28.65	.0000
Time	2	29.91	.0000
Media	2	10.47	.0053
Treatment	2	10.63	.0049
Likelihood ratio	48	38.36	.84

of the study site one week after the second spray. No genetically engineered P. syringae was detected in any off-site vegetation samples.

Quality Control Samples

Out of 120 isolates of known identity submitted to UCD for gene probe analysis, 96 isolates (80%) were correctly identified, 13 isolates (10.8%) were incorrectly identified, and the identity of 11 out of 80 isolates (13.8%) of genetically engineered bacteria could not be confirmed (Table 6). Isolates of genetically engineered P. syringae were correctly identified (87.5%, n=40) more frequently than either isolates of genetically engineered P. fluorescens (67.5%, n=40) or strains of non-engineered bacteria (85.0%, n=40). Most of the incorrect identification of isolates through gene probe analysis occurred after the first but not after the second Frostban® application. This was likely due to the fact that the correct probe for P. fluorescens was not available to U.C.D. until the second release.

The species intentionally applied were detected and correctly identified through classical diagnostic tests in 96.3% of the samples (n= 54) (Table 7), where genetically engineered Pseudomonas species were used to inoculate vegetation to create matrix spike samples. On one sampling date (14 days after the second spray), both species of Frostban® bacteria were recovered from two samples even though only genetically engineered P. fluorescens was intentionally applied. Results from gene probe analysis for isolates from these 2 samples conformed to preliminary diagnostic test results indicating that contamination of these two samples may have occurred during sample preparation. In general, confirmation of the identify of Frostban® bacteria recovered from matrix spike samples through gene probe analysis was less consistent than species identification

Table 6. Frequency of correct, incorrect and uncertain identification by the UC Davis Plant Pathology Laboratory of bacterial isolates submitted as quality control samples for the spring, 1987 Frostban® Monitoring Study.

Test Isolate Identity	Sampling Date 4/10/87	4/24/87	5/1/87	5/8/87	5/13/87	5/19/87	5/26/87	6/2/87	6/8/87	Total
Correctly Identified										
P. syringae	9/9	2/4	1/3	4/4	3/4	4/4	4/4	4/4	4/4	35/40 (87.5%)
P. fluorescens	6/9	4/4	1/3	0/4	4/4	4/4	0/4	4/4	4/4	27/40 (67.5%)
Other	9/9	4/4	1/3	1/4	3/4	4/4	4/4	4/4	4/4	34/40 (85.0%)
Total	24/27 (88.9%)	10/12 (83.3%)	3/9 (33.3%)	5/12 (41.7%)	10/12 (83.3%	12/12 (100%)	8/12 (66.6%)	12/12 (100%)	12/12 (100%)	96/120 (80%)
Incorrectly Identified										
P. syringae	0/9	0/4	1/3	0/4	0/4	0/4	0/4	0/4	0/4	1/40 (2.55%)
P. fluorescens	0/9	0/4	2/3	4/4	0/4	0/4	0/4	0/4	0/4	6/40 (15.0%)
Other	0/9	0/4	2/3	3/4	1/4	0/4	0/4	0/4	0/4	6/40 (15.0%)
Total	0/9 (0%)	0/4 (0%)	5/9 (55.5%)	7/12 (58.3%)	1/12 (8.3%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	13/120 (10.8%)
Undetermined										
P. syringae	0/9	2/4	1/3	0/4	1/4	0/4	0/4	0/4	0/4	4/40 (10.0%)
P. fluorescens	3/9	0/4	0/3	0/4	0/4	0/4	4/4	0/4	0/4	7/40 (17.5%)
Total	3/18 (16.6%)	2/8 (25.0%	1/6 (16.6%)	0/4 (0%)	1/8 (12.5%)	0/8 (0%)	4/8 (50.0%)	0/8 (0%)	0/8 (0%)	11/80 (13.8%)

Table 7. Number of spiked strawberry foliage samples[1] from which isolates of Pseudomonas species were correctly identified by the UC Davis Plant Pathology Laboratory during spring, 1987, using gene probe analysis.

	Sampling Date									Frequency of Positive Identification
	4/10/87	4/24/87	5/1/87	5/8/87	5/13/87	5/19/87	5/26/87	6/2/87	6/8/87	
Single spp. spike										
P. syringae	2	2	2	2	2	2	2	2	2	100%
P. fluorescens	0	2	1	1	2	2	2	2	2	77%
Double spp. spike										
P. syringae	1	2	1	2	0	2	2	2	---	75%
P. fluorescens	0	1	1	1	1	1	1	2	4	60%

1. Out of 2 isolates for each sample type per sampling date excepting 6/8/87 when there were 4 positive isolates of P. fluorescens submitted.

Table 8. Number of spiked strawberry foliage quality control samples from which Pseudomonas species were isolated and correctly identified on each sampling date by the CDFA Analysis and Identification Laboratory during spring, 1987.

	Sampling Date									Frequency of Positive Identification
	4/10/87	4/24/87	5/1/87	5/8/87	5/13/87	5/19/87	5/26/87	6/2/87	6/8/87	
Single spp. spike										
P. syringae	2	2	2	2	2	2	2	2	2	100%
P. fluorescens	2	2	2	2	2	2	2	2	2	100%
Double spp. spike										
P. syringae	2	2	2	2	2	2	2	2	0	88.9%
P. fluorescens	2	2	2	2	2	2	2	2	2	100%

1. Out of 2 samples for each sample type per sampling date.

using classical diagnostic tests (Table 8). P. syringae applied alone to strawberry vegetation was always isolated and correctly identified through gene probe analysis. However, the presence of genetically engineered P. syringae was confirmed less often (75% positive confirmation) when isolates were submitted for analysis from samples spiked with both species of Frostban® bacteria. P. fluorescens applied alone to strawberry foliage was identified less often (77% positive confirmation) than comparable isolates from foliage treated with P. syringae, and there were a higher number of incorrect isolate identifications; 7 out of 54 isolates identified as P. syringae were isolates of P. fluorescens. The presence of genetically engineered P. fluorescens was confirmed less often (60% positive confirmation) when isolates from samples spiked with both species of Frostban® bacteria were submitted for gene probe analysis, than when P. fluorescens was applied alone. There appeared to be an increase in positive confirmation over time of Frostban® bacteria used in matrix spike samples, with a high frequency of positive confirmations occurring after the second spray date as compared with positive confirmations after the first.

IV. DISCUSSION

There did not appear to be any general patterns of association between wind direction during the two Frostban® applications and the location of off-site recoveries. The higher levels of off-site recovery of genetically engineered bacteria during the second spray may be explained by the higher wind speeds during the second versus the first spray. The actual pattern of detection is probably the result of multiple factor interactions including placement of experimental treatments and temporal coincidence of application location and weather events such as wind direction and intensity. Even at wind speeds of

less than 0.8 m/s, dispersal of both species of genetically engineered bacteria in aerosol form was common during application to 15 m off-site.

Following the first application of genetically engineered bacteria, there was a linear decline over time in the $\log_e$ abundance index of Frostban® bacteria recovered from treated plots. It is likely that the high temperatures and low relative humidities recorded during the course of this study were responsible for the steady and relatively rapid declines in abundance of bacteria observed. A similar reduction in the abundance of Pseudomonas syringae with the onset of hot, dry weather has been reported in pear orchards (Lindow, 1982). The second application, 19 days after the first, did not, in general, increase the abundance of Frostban® bacteria to levels detected after the first application. The abundance of genetically engineered P. syringae was significantly higher than that of genetically engineered P. fluorescens over the course of the study although the rate of decrease of both species over time was not significantly different. This difference in abundance might have been due to either unequal amounts of these two species applied initially, or differential response to environmental conditions immediately following application. The recovery of total fluorescent Pseudomonads (fluorescent bacteria recovered on media amended with cycloheximide only) was consistently higher than that of genetically engineered bacteria, although bacteria of both types decreased over time. This fact supports the conclusion that the decline in abundance of Frostban® bacteria over time was the direct result of generally unfavorable weather conditions.

A high percentage (80%) of isolates of known identity were correctly identified using genetic analysis. Most of the incorrect identification of isolates using this technique occurred after the first Frostban application but not after the

second. There were difficulties with DNA extraction using the initial extraction protocol supplied by AGS. A modified protocol was utilized for samples collected after the second Frostban® application. It is likely that improvement in laboratory methods accounts for the greater accuracy of gene probe analyses for samples collected after the second spray.

Genetically engineered bacteria were virtually absent from off-site vegetation samples. From a total of 193 samples taken from perimeter oat plants, only one contained genetically engineered bacteria. Sampling of apricot trees, tomatoes, corn, weeds, and vegetation from pear orchards to a distance of approximately 90m yielded no positive samples. Few genetically engineered bacteria were detected in plots which had not been treated with the bacteria. Frostban® bacteria were detected in a high percentage of air samples taken at the perimeter of the buffer zone 15 m off-site but not in most of the oat samples taken at the same distance. Either the concentration of Frostban® bacteria in air dispersing off-site during applications was low enough that impaction of perimeter oat leaves and other off-site vegetation was a rare event, or unfavorable weather conditions made colonization by inoculum reaching perimeter plants unlikely.

V. REFERENCES

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APPENDIX I

LABORATORY ANALYTICAL METHODS

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Bacterial Media and Solutions

To prepare reagent stock solutions, 5.0 g of cycloheximide or 2.5 g of rifampicin were added to 50 ml 95% ethanol for cycloheximide and 100 ml DMSO for rifampicin.

King's B medium was prepared by adding 18.0 g of prepared media to 1000 ml of distilled water adding 10 ml of glycerol and steam sterilizing (121°C; 15 lbs.; 20 min.).

To prepare King's B medium with antibiotics, 1.5 ml stock cycloheximide and/or 3 ml stock rifampicin were added to King's B medium following steam sterilization. This amended media contained 150 ppm cycloheximide and 75 ppm rifampicin, or 150 ppm cycloheximide only, with approximately 15 ml media per culture plate. King's B medium amended with rifampicin was used within 7 to 10 days after preparation to avoid problems associated with antibiotic degradation.

Preparation of $CuSO_4$ media, amended with cycloheximide and rifampicin, is similar to the above procedure with 0.798 g of anhydrous $CuSO_4$ added prior to autoclaving (5 Mm $CuSO_4$). A concentration of 5.0 Mm $CuSO_4$ is believed to make the media selective for the copper resistant strain of P. fluorescens applied by AGS.

The phosphate buffer (0.01M) was prepared by adding 1.74 g of potassium phosphate dibasic (Trihydrate) and 1.37 g of potassium phosphate monobasic to 1000 ml of distilled water, and adjusting the pH to 7.0.

The sodium-potassium peptone buffer was prepared by adding 10.95 g sodium phosphate dibasic heptahydrate, 3.5 g potassium phosphate monobasic, and 1.0 g Bacto peptone to 1000 ml of distilled water, and adjusting the pH to 7.1 and then steam sterilizing.

Nutrient broth amended with rifampicin was prepared by adding 8.0 g of Difco nutrient broth to 1000 ml of distilled water, steam sterilizing, and then adding 1 ml of rifampicin stock solution.

Luria broth media was made by adding 5 g of yeast, 10 g tryptone, and 5 g NaCl to 600 ml of deionized water. The pH was then adjusted to 7.5 with 1N NaOH and the volume increased to 930 ml with deionized water.

Oxidase Test

This test is particularly useful for differentiating pseudomonads from certain other Gram negative rods. The oxidase test is an indirect test for the presence of a cytochrome of the "C" type in the respiratory transport chain.

Required:

1. Filter paper.
2. 1.0% aqueous solution of N, N, N', N'-tetramethyl-p- phenylenediamine dihydrochloride.
3. 24 hour bacterial culture.

Procedure:

1. Spread a loop of bacteria on a piece of filter paper soaked in 1.0% aqueous solution of N, N, N', N'-tetramethyl-p-phenylenediamine dihyrochloride. Production of a purple color in 5-10 seconds is considered a positive test; no color is a negative test.

2. P. syringae is oxidase negative, and P. fluorescens is oxidase positive.

Arginine Dihydrolase Test

Arginine dihydrolase, present in certain bacteria, permits the conversion of arginine into ornithine, ammonia, and carbon dioxide with the production of ATP under anaerobic conditions.

Required:

1. 3 ml of Thornley's medium 2A.
2. 5 ml screw cap bottle.
3. 48 hour culture.

Procedures:

1. Pour about 3 ml media into 5 ml bottle.
2. Autoclave and let cool.
3. Stab inoculate the bacteria.
4. Seal the stab with sterile vaseline.
5. Incubate at room temperature for 3-4 days.
6. Note color change of the phenol red indicator. A pH change to the alkaline side (red) due to the ammonia is regarded as positive for arganine dihydrolase.
7. P. syringae is arginine negative, and P. fluorescens arginine positive.

Reference:

1. Thornely, M. J. 1960. The differentiation of Pseudomonas from other gram negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23:37-52.

Ice Nucleation Test

Ice nucleation is a characteristic of most wild type Pseudomonas bacteria. Frostban® bacteria lack the ability to act as a nucleus for ice formation.

Required:

1. Cooling chamber at -9°C.
2. Aluminum foil boats coated with xylene paraffin.
3. Culture to be tested.
4. Sterile toothpicks.
5. Dust free (covered) ELISA plates.
6. Multichannel automatic pipetter.

Procedures:

1. Lift cultures to be tested using a sterile toothpick.
2. Prepare a culture suspension in the well of the ELISA plate in 200 ul of sterile water, by using the toothpick as a disposable transfer tool.
3. Prepare a foil boat and float it on the cooling chamber.
4. Transfer a 20 ul drop of bacterial suspension to the cool foil using the automatic pipetter.
5. Allow 1-2 minutes for the droplets to freeze solid.
6. Check droplets with a sterile toothpick to determine if frozen.
7. About ten replicate droplets should be tested for each isolate to confirm or negate ice nucleation ability.

References:

1. Lindow, S. 1987. Personal communication.

RFLP Analysis of Recombinant Bacteria

1. Prepare Probe

A. Extract and purify probe DNA(s), determine concentration(s)
B. Standardize concentrations for multi-isolate probes
C. Label probe DNA with [^{32}P]dCTP and purify
D. Store at -20c until used.

2. Extract DNA of Interest

Genomic, chromosomal, plasmid; based on recombination of interest
ICE(-): extract genomic (total) DNA

3. Restriction Enzyme Digest

A. Digest known concentration of DNA with appropriate restriction endonucleases at 2 units enzyme/ug DNA
ICE(-): 1. Digest aliquot of ALL isolates with Pvu II = Eco RI
2. Digest aliquot of isolates which yield inconclusive results upon initial digest analysis;
suspected P. syringae: Bgl II + Eco RI
suspected P. fluorescens: Kpn I + Sal I
B. Stop digestion by addition of gel loading buffer:
1 vol. glycerol + 1 vol. 250mM EDTA, pH8 +
1 mg/ml SDS + 0.25% Bromophenol Blue dye

4. Electrophoresis

A. Gel= Agarose (0.7%) in 1x TBE buffer
B. Running buffer= 1x TBE
C. Load 1-3 ug of each DNA isolate onto gel (5 ul)
D. Run at 10 volts/cm (100V on 10 cm plate)

NOTE: Must run digested isolates of known identity as standards.

5. Southern Blot

A. Capillary transfer DNA to NYTRAN membrane
(Schleicher & Schuell) charge modifed nylon
B. Gel Prep: dupurinate (0.25N HC1, 8 min.)
Denature (0.5 N NaOH + 1M NaC1, twice @ 12 min.)
Neutralize (0.5 M Tris, pH 7.5 + 1.5M Nac1, twice @ 12 min.)
C. Blot buffer= 10x SSPE
D. Blot overnight(>/= 8 hr), rinse in 2x SSPE
E. Fix DNA to membrane by UV crosslinking: 5 min., 302nm
F. Store blots desiccated, at 4c until probed.

6. Probe Blots

A. Prehybridize >/= 3 hr, 43c, agitated
B. Hybridize >/= 8 hr (usually overnight), 43c, agitated with [^{32}P] labelled DNA at $5x10^6$ cpm/ml solution
C. Wash blot(s) to remove unhybridized probe.

7. Autoradiograph Blots

A. Monitor blot(s) to determine length of film exposure (rough estimate with geiger counter)
B. Expose film (Kodak XAR-5) required time
C. Reexpose as required for suitable sensitivity.

8. Analyze Results

A. Compare RFLP pattern of unknowns to those of standards to evaluate identity
B. Perform secondary restriction digest analaysis of aliquots from samples as necessary to determine identify of isolates.

Pseudomonas Total DNA Extraction

1. Culture: ~5 ml LB (or equivalent), 28c, overnight (> 18hr)

2. Recover Cells: Spin ~800-900 g, 10 min, wash ~1 ml TE, pH 8
   Spin as above, resuspend in 250 ul TE

3. Lyse: Add 250 ul stock solution:
   235.5 ul TE + 12.5 ul 20% SDS + 2 ul Proteinase K
   Invert to mix; incubate 65c, 30 min, with agitation
   Cool to ~37C

4. Removal of RNA: Add 10 ul RNASE A stock
   (Heat RNASE to 100c, 15 min immediately prior touse)
   Incubate 37c, 30 min, with agitation
   Shear DNA by passage 10-12X through P-1000 pipette tip
   (Helps disociate DNA from proteinaceous matrix)

5. Phenol-Chloroform Extraction: Add 500 ul reagent:
   Extract twice @ phenol: ChC13, followed by once @ ChC13
   Mix by inversion, spin ~12000 rpm, 10 min.
   Remove aqueous phase to new tube
   (Transfer with wide-mouthed pipette helps avoid proteinaceous contaminants)

6. Ethanol Precipitation: Initial DNA vol. ~<= 350 ul
   Add 7.5M ammonium acetate to 2M (vol./2.75)
   Add 2 vol. 100% EtOH @ -20c, mix by inversion
   Incubate -70c, >= 15 min, spin ~12000 rpm, 20 min, 4c
   Discarb supernatant, wash ~500 ul 70% EtOH @ -20c
   Drain tube, speed-vac just to dryness (~3 min)
   Resuspend in desired vol. TE

7. Storage: Store @ -20c in small aliquots

8. Reagents:

   TE 10 mM Tris-C1, pH 8.0
   1mM EDTA, pH 8.0

   Phenol: Chloroform 1:1 mixutre of
   a) phenol: equilibrated with 0.1M Tris, pH 8
      + 0.2% B-mercaptoethanol + 0.1% hydroxyquinoline
   b) ChC13: 24:1 chloroform: isoamyl alcohol

   RNASE A 10 mg/ml in 10mM Tris-C1, pH 7.5 + 15mM NaC1
   Heat to 100c, 15 min; cool slowly to RT
   Aliquot, store -20c

   Proteinase K 20 mg.ml in dH_2O
   Aliquot, sotre -20c

Membrane Hydridization

1. Prehybridization

Component	10ml	8ml	5ml
20% SSPE	750ul	600ul	375ul
20% SDS	500ul	400ul	250ul
PAES (#)	200ug	160ug	100ug
dH_2O	3.25ml	2.60ml	1.625ml
formamide (DI)	5.0ml	4.0ml	2.5ml
S.S. DNA	500ul	400ul	250ul

Mix all components except DNA first
Denature DNA just prior to addition; heat @ 100c, 5 min
maintain DNA denatured by immediately:
  plunging into ice or diluting into prehydridized solution
Add prehyb. solution to membrane(s) in seal-a-meal pouch
  exclude air from pouch and seal
Incubate: 49c, > 2 hr, with agitation (*)

2. Hybridizaton

Component	10ml	8ml	5ml
20% SSPE	750ul	600ul	375ul
20% SDS	500ul	400ul	250ul
PAES (#)	200ug	160ug	100ug
dH_2O	1.15ml	920ul	575ul
formamide (DI)	5.0ml	4.0ml	2.5ml
50% PEG	2.0ml	1.6ml	1.0ml
S.S. DNA	500ul	400ul	250ul

probe DNA ~>= 2-5 X 10^6 CPM/ml of solution

Mix all components except DNAs first; prewarm to 49c
Shear and Denature probe and S.S. DNA prior to addition
  in 100ul 0.2N NaOH (2% @ 10N), in microfuge tube
  vortex, centrifuge to consolidate, heat @ 100c, 5 min
Add probe immediately to hybrid solution
Remove prehybridized membrane(s) into fresh seal-a-meal pouch
Add hybrid solution to pouch, exclude air, and seal
Incubate: 49c, >= 3 hr, with agitation (**)

Temperature and Time of incubation are determined by:

(*) Temp = 16.6(log[Na+])+0.41(%[G+C]+81.5-0.65(% formamide)-(500/probe length)

= 16.6(log[0.27])+0.41(53)+81.5-0.65(50)-(500/600) = 61
criterion = Temp-12 = 61-12 = 49C

(**) Cot 1/2 = (1/ug DNA) (Kb probe/5) (rxn vol/10) (2)

1/2 = (1/0.25) (0.6/5) (10/10) (2) = 0.96 hr
criterion >= (3) (Cot 1/2) >= 2.88 hr

(#) PAES: Polyanetholesulfonic acid (a synthethic Heparin analog)
Sodium salt: Calbiochem # 528862

Post-Hybridization Washing

STEP	SOLUTION	20% SSPE	20% SDS	H2O	TIME	TEMP
rinse	2xSSPE	10ml		90ml	~1min	RT
wash 1	2xSSPE+0.1%SDS	25.000	1.25ml	223.75	5min	RT
wash 2	0.5xSSPE+0.1%SDS	6.250	1.25ml	242.50	5min	RT
wash 3	0.1xSSPE+1.0%SDS	1.250	12.50	236.25	15min	68c
rinse	0.1xSSPE	1.250		248.75	5min	RT

Note: initial rinse performed in plastic dish
subsequent wash/rinse in "omni blot"
double each wash volume when using larger pouch

Blot briefly to Whatman 3mm

Wrap in saran

Monitor cpm of each membrane to determine length of exposure for autoradiography

Post-Hybridization Probe Removal

For Removal of Labelled Probe from Nylon Membranes

Note: Membranes must be kept moist prior to stripping
Store Blots for reuse only after stripping
Higher [formamide] +/or Temp +/or incub. Time may be necessary for removal of some probes
Colorimetric probes or substrates cannot be removed

Components	Volume	Conditions
Method A:		
6x SSPE	15ml @ 20x	Incubate: 65C
50% Formamide	25ml	30 min,
H_2O	10ml	agitate
Rinse briefly in ~50ml 2x SSPE		
Method B:		
5mM Tris-Cl, pH 8	125ul @ 2M	Incubate: 65C
0.2mM EDTA	40ul @ 250mM	1-2 hr, agitate
0.05% pyrophosphate		
100ug PAES		
H_2O	49.835ml	
Rinse briefly in ~50ml 1x SSPE		

Probe Preparation

1. Plasmid DNA

   pRLG12 + pMWS5 recombinant plasmids derived from Pseudomonas syringae and P. fluorescens respectively, extracted and purified from E. coli host strains, resuspended in a mixture: 7.1 pRLG12 : 8.1 pMWS5.

2. Linear DNA

   Plasmid DNA was cut with Eco RI + Hind 3 simultaneously resulting fragments = recombinant insert & vector remnants purified by phenol-chloroform extraction and ethanol ppt. resuspended at 100ng/ul.

3. Probe

   46.7ng pRLG12 + 53.29ng pMWS5 = 100ng = 1 ult stock
   labelled via random primer extension
   with [32] dCTP at 3000 Ci/mmol; 10 mCi/ml (Amersham)
   to a specific activity of approximately 10^9 cpm/ug
   purified by ethanol ppt.

   Probe activity in hybridization solution = 5 $X10^6$ cpm/ml
   Blots were incubated in 8ml solution, overnight.

Plasmid Isolation Procedure

Modified from Promega Catalog procedure from H.C. Birnboim and J.Doly, Nucl. Acids Res. 7:1513:1523, "A rapid alkaline extraction procedure for screening recombinant plasmid DNA."

1. Inoculate 50ml of LB media with antibiotic (e.g. 100 ug/ml ampicilling) and grow cells overnight (14 or 15 hours) at 37c with shaking.

2. Cool the cultures on ice. Collect cells by centrifugation at 5000 rpm for 10 minutes at 4c.

3. Resuspend cells in 1.65 ml of solution I (pH approx. 8)
   Solution I is 50mM glucose
   25mM Tris, 15mM HC1
   10 mM Na_2EDTA

4. Add 1.65 ml of Solution I inwhich lysozyme was freshly dissolved to a final concentration of 10 mg/ml. Mix the suspension by gentle swirling.

5. Hold the suspension on ice for 20 minutes.

6. Add 6.7 ml of fresh Solution II, prepared from roughly standardized NaOH and 10% SDS. Mix the suspension by inversion.
   Solution II is 0.2 M NaOH
   1% SDS

7. Hold the suspension on ice for 10 minutes.

8. Add 5ml of 5M KAc, 5M acetic acid (pH 4.8). Mix the suspension by inversion.

9. On ice, 20 minutes.

10. Centrifuge the suspension at 15,000 rpm for 15 minutes at 4C.

11. Remove the supernatant, transferring it to a new tube. Avoid transferring flecks of the white precipitate, using filtration through Miracloth.

12. Add 0.6 volumes of isopropanol.

13. Hold at room temperature for 15 minutes, no longer.

14. Spin at 10,000 rpm for 30 minutes at 21c.

15. Remove the supernatant. Wash the precipitate with 70% ethanol.
    Dry the tube walls by inverting the tubes on pads of Kimwipes.

Random Primed DNA Labeling (BMB Kit)

1. Denature DNA
   Aliquot DNA and water into microfuge tube
   Heat DNA 10 min, 95C; or 5 min 100C (boiling bath)
   Cool on ice water

2. Reaction Mix

COMPONENT	1X	2X	4X	Notes
NDA	25ng	50ng	100ng	Vol varies
pLUC2	0.125ul	0.250ul	0.500ul	pLUC2=200ng/ul
SDW	8.875ul	17.75ul	35.5ul	using pLUC2
cold dNTP's	3ul	6ul	12ul	dATP+dTTP+dGTP
rxn mix	2ul	4ul	8ul	hexanucl. +buf.
[32P] dCTP	50uCi	100uCi	200uCi	3000Ci/mmol
@ 10 mCi/ml	5ul	10ul	20ul	
Klenow enzyme	1ul	2ul	4ul	DNA polymerase
TOTAL VOLUME	20ul	40ul	80ul	

3. Incubate
   >/= 30 min, 37c (water bath is best)

4. Stop Reaction and Clean-Up Probe

COMPONENT	20ul	40ul	80ul	Notes
250mM EDTA	8ul	12ul	16ul	To 20mM
SDW	22.83ul	34.25ul	25.7ul	To volume
.5ug/ul ssDNA	20ul	20ul	20ul	10ug as carrier
20% SDS	2.5ul	3.75ul	5ul	To 0.5%
7.5M NH_4OAc	26.67ul	40ul	53.3ul	To 2M
EtOH	200ul	300ul	400ul	2 olumes

   incubate: -70C > 15 minutes
   centrifuge: ~12,000 rpm, 20 minutes, 4c (microfuge)
   decant supernatant, drain tube
   wash pellet with ~500ul 70% EtOH, -20c; decant wash and drain
   dry pellet: speed vac ~3 minutes

5. Resuspend Probe
   100ul TE, pH 7.5-8.0
   heat to 37c, ~10 minutes to help dissolve, vortex briefly

6. Measure Activity
   (not true activity, but estimate based on cpm/ul)
   aliquot 0.5ul into each of three scintillation vials
   measure cpm withfull window, 2-3x each vial
   mean all values; 2x mean = cpm/ul

Diagnostic Test Profile for Genetically-Engineered *Pseudomonas* Species

	P. syringae	*P. fluorescens*
Fluorescense	+	+
Oxidase	-	+
Arginine Dihydrolase	-	+
Ice Nucleation	-	-
Positive Identification through gene probe analysis	+	+

APPENDIX II

DIAGNOSTIC TEST RESULTS, FINAL DIAGNOSES AND ABUNDANCE OF BACTERIA FROM FIELD SAMPLES

APPENDIX II

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
A			4/10/87	76	LEA	0	1						N												
A			4/24/87	299	LEA	0	2						N												
A			5/01/87	499	LEA	0	1						N												
A			5/08/87	599	LEA	0	3						N												
A			5/13/87	699	LEA	0	3						N												
A			5/19/87	799	LEA	0	1						N												
A			5/26/87	899	LEA	0	1						N												
A			6/02/87	976	LEA	0	1						N												
A			6/09/87	1076	LEA	0	1						N												
C			4/10/87	38	LEA	0	1						N												
C			4/10/87	55	LEA	0	1						N												
C			4/24/87	227	LEA	0	4						N												
C			4/24/87	291	LEA	0	1						N												
C			5/01/87	421	LEA	0	2						N												
C			5/01/87	451	LEA	0	4						N												
C			5/08/87	521	LEA	0	3						N												
C			5/08/87	551	LEA	0	3						N												
C			5/13/87	621	LEA	0	4						N												
C			5/13/87	651	LEA	0	4						N												
C			5/19/87	721	LEA	0	1						N												
C			5/19/87	751	LEA	0	2						N												
C			5/26/87	821	LEA	0	3						N												
C			5/26/87	851	LEA	0	4						N												
C			6/02/87	938	LEA	0	3						N												
C			6/02/87	955	LEA	0	4						N												
C			6/09/87	1038	LEA	0	0						N												
C			6/09/87	1055	LEA	0	4						N												
F			4/10/87	59	LEA	0	4						N												
F	1		4/10/87	82	FLO	0	2						N												
F	1		4/24/87	288	LEA	3	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	1		5/01/87	450	LEA	2	3	+	+	+	-	S	F	+	+	+	-	S	F	+	+	+	-	S	F
F	1		5/08/87	550	LEA	1	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	N	F
F	1		5/13/87	526	LEA	2	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	1		5/19/87	726	LEA	2	2	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	1		5/26/87	826	LEA	1	2	+	+	+	-	N	F	+	+	+	-	F	F	+	+	+	-	N	F
F	1		6/02/87	982	LEA	0	1						N												

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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
F	1		6/09/87	1082	LEA	0	4						N												
F	2		4/10/87	7	FLO	0	3						N												
F	2		4/10/87	85	LEA	0	3						N												
F	2		4/24/87	230	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	2		5/01/87	481	LEA	2	3	+	-	-	-	S	S	+	-	-	-	F	S	+	-	-	-	S	S
F	2		5/08/87	581	LEA	2	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	2		5/13/87	593	LEA	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	2		5/19/87	793	LEA	1	2	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	2		5/26/87	893	LEA	1	1	+	-	-	-	N	S	+	-	-	-	S	S	+	-	-	-	S	S
F	2		6/02/87	959	LEA	0	1						N												
F	2		6/09/87	1050	LEA	0	4						N												
F	3		4/10/87	17	LEA	0	2						N												
F	3		4/10/87	22	FLO	0	3						N												
F	3		4/24/87	279	LEA	0	4						N												
F	3		5/01/87	447	LEA	0	2						N												
F	3		5/08/87	547	LEA	0	2						N												
F	3		5/13/87	583	LEA	0	2						N												
F	3		5/19/87	783	LEA	0	2						N												
F	3		5/26/87	883	LEA	0	1						N												
F	3		6/02/87	907	LEA	0	1						N												
F	3		6/09/87		LEA	0	4						N												
F	4		4/10/87	68	FLO	0	3						N												
F	4		4/10/87	100	LEA	0	2						N												
F	4		4/24/87	235	LEA	0	1						N												
F	4		5/01/87	455	LEA	0	3						N												
F	4		5/08/87	555	LEA	0	2						N												
F	4		5/13/87	580	LEA	0	2						N												
F	4		5/19/87	780	LEA	0	1						N												
F	4		5/26/87	880	LEA	0	1						N												
F	4		6/02/87	985	LEA	0	1						N												
F	4		6/09/87	1085	LEA	0	1						N												
F	5		4/10/87	12	FLO	0	4						N												
F	5		4/10/87	34	LEA	0	3						N												
F	5		4/24/87	271	LEA	0	4						N												
F	5		5/01/87	471	LEA	0	4						N												
F	5		5/08/87	571	LEA	0	4						N												
F	5		5/13/87		LEA	0	3						N												

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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
F	5		5/19/87	768	LEA	0	2						N												
F	5		5/26/87	868	LEA	0	1						N												
F	5		6/02/87	922	LEA	0	1						N												
F	5		6/09/87	1022	LEA	0	1						N												
F	6		4/10/87	70	FLO	0	4						N												
F	6		4/10/87	87	LEA	0	2						N												
F	6		4/24/87	256	LEA	3	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6		5/01/87	427	LEA	2	3	+	+	+	-	S	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6		5/08/87	527	LEA	2	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6		5/13/87	563	LEA	1	1	+	+	+	-	N	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6		5/19/87	763	LEA	0	1																		
F	6		5/26/87	863	LEA	0	1	+	+	+	-	N	F	+	+	+	-	N	F	+	+	+	-	N	F
F	6		6/02/87	917	LEA	0	1																		
F	6		6/09/87	1017	LEA	0	1																		
F	7		4/10/87	29	LEA	0	3						N												
F	7		4/10/87	93	FLO	0	4						N												
F	7		4/24/87	239	LEA	0	3						N												
F	7		5/01/87	413	LEA	0	2						N												
F	7		5/08/87	513	LEA	0	3						N												
F	7		5/13/87	503	LEA	0	1						N												
F	7		5/19/87	734	LEA	0	1						N												
F	7		5/26/87	834	LEA	0	1						N												
F	7		6/02/87	968	LEA	0	1						N												
F	7		6/09/87	1068	LEA	0	4						N												
F	8		4/10/87	45	FLO	0	4						N												
F	8		4/10/87	86	LEA	0	2						N												
F	8		4/24/87	267	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	8		5/01/87	500	LEA	3	3																		
F	8		5/08/87	600	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	8		5/13/87	511	LEA	1	2	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	8		5/19/87	762	LEA	2	2	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	8		5/26/87	862	LEA	1	2	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	8		6/02/87	1000	LEA	0	1						N												
F	8		6/09/87	1100	LEA	0	1						N												
F	9		4/10/87	2	FLO	0	2						N												
F	9		4/10/87	74	LEA	0	4						N												
F	9		4/24/87	260	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	9		5/01/87	440	LEA	3	3	+	-	-	-	F	S	+	-	-	-	F	S	+	-	-	-	S	S

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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

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F	9		5/13/87	507	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	9		5/08/87	539	LEA	2		+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	9		5/19/87	770	LEA	2	2	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	9		5/26/87	870	LEA	1	1	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	9		6/02/87	912	LEA	0	1						N												
F	9		6/09/87	1012	LEA	0	1						N												
F	10		4/10/87	31	LEA	0	2						N												
F	10		4/10/87	41	FLO	0	4						N												
F	10		4/24/87	263	LEA	0	1						N												
F	10		5/01/87	412	LEA	0	2						N												
F	10		5/08/87	512	LEA	0	3						N												
F	10		5/13/87	594	LEA	0	1						N												
F	10		5/19/87	794	LEA	0	1						N												
F	10		5/26/87	894	LEA	0	1						N												
F	10		6/02/87	934	LEA	0	1						N												
F	10		5/09/87	1034	LEA	0							N												
F	11		4/10/87	28	FLO	0	2						N												
F	11		4/10/87	97	LEA	0	4						N												
F	11		4/24/87	351	LEA	2	3	+	+	+	-	N	F	+	+	+	-	F	F	+	+	+	-	F	F
F	11		5/01/87	482	LEA	2	3	+	+	+	-	N	F	+	+	+	-	S	F	+	+	+	-	S	F
F	11		5/08/87	582	LEA	1	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	11		5/13/87	520	LEA	0	3						N												
F	11		5/19/87	720	LEA	1	2	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	11		5/26/87	820	LEA	1	1	+	+	+	-	N	F	+	+	+	-	N	F	+	+	+	-	N	F
F	11		6/02/87	970	LEA	0	1						N												
F	11		6/09/87	1070	LEA	0	1						N												
F	12		4/10/87	23	FLO	0	4						N												
F	12		4/10/87	48	LEA	0	1						N												
F	12		4/24/87		LEA	0	1						N												
F	12		5/01/87	458	LEA	0	1						N												
F	12		5/08/87	595	LEA	0	3						N												
F	12		5/13/87	574	LEA	0	2						N												
F	12		5/19/87	774	LEA	0	2						N												
F	12		5/26/87	874	LEA	0	1						N												
F	12		6/02/87	987	LEA	0	1						N												
F	12		6/09/87	1087	LEA	0	1						N												
F	13		4/10/87	32	LEA	0	2						N												
F	13		4/10/87	69	FLO	0	2						N												

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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
F	13		4/24/87	313	LEA	0	1						N												
F	13		5/01/87	495	LEA	0	2						N												
F	13		5/08/87	558	LEA	0	3						N												
F	13		5/13/87	531	LEA	0	1						N												
F	13		5/19/87	731	LEA	0	1						N												
F	13		5/26/87	831	LEA	0	2						N												
F	13		6/02/87	993	LEA	0	1						N												
F	13		6/09/87	1093	LEA	0	4						N												
F	14		4/10/87	81	FLO	0	3						N												
F	14		4/10/87	88	LEA	0	2						N												
F	14		4/24/87	337	LEA	0	2						N												
F	14		5/01/87	487	LEA	0	4						N												
F	14		5/08/87	587	LEA	0	3						N												
F	14		5/13/87	506	LEA	0	3						N												
F	14		5/19/87	800	LEA	0	1						N												
F	14		5/26/87	900	LEA	0	2						N												
F	14		6/02/87	929	LEA	0	1						N												
F	14		6/09/87	1029	LEA	0	1						N												
F	14		4/10/87	43	FLO	0	2						N												
F	15		4/10/87	92	LEA	0	2						N												
F	15		4/24/87	355	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	F	S
F	15		5/02/87	464	LEA	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	15		5/08/87	564	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	15		5/13/87	542	LEA	2	2	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	15		5/19/87	742	LEA	2	2	+	-	-	-	S	S	+	-	-	-	N	S	+	-	-	-	S	S
F	15		5/26/87	842	LEA	1	1	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	15		6/02/87	945	LEA	1	1	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	15		6/09/87	1045	LEA	0	1						N												
F	16		4/10/87	13	LEA	0	2						N												
F	16		4/10/87	75	FLO	0	3						N												
F	16		4/24/87	359	LEA	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
F	16		5/01/87	486	LEA	2	2	+	+	+	-	S	F	+	+	+	-	S	F	+	+	+	-	S	F
F	16		5/08/87	586	LEA	1	2	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	16		5/13/87	566	LEA	1	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	16		5/19/87	766	LEA	0	2						N												
F	16		5/26/87	866	LEA	0	1						N												
F	16		6/02/87	986	LEA	0	1						N												
F	16		6/09/87	1086	LEA	0	1						N												

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Diagnostic test results, final diagnoses and abundance of luorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
0	1	4/24/87	234	LEA	0	2							N												
0	1	4/25/87	352	LEA	1	0							N												
0	1	5/01/87	429	LEA	0	0							N												
0	1	5/13/87	629	LEA	0	4							N												
0	1	5/19/87	650	LEA	0	1							N												
0	2	4/24/87	244	LEA	0	1							N												
0	2	4/25/87	304	LEA	0	1							N												
0	2	5/01/87	402	LEA	0	4							N												
0	2	5/13/87	602	LEA	0	1							N												
0	2	5/19/87	648	LEA	0	1							N												
0	3	4/24/87	280	LEA	0	1							N												
0	3	4/25/87	325	LEA	0	1							N												
0	3	5/01/87	432	LEA	0	1							N												
0	3	5/13/87	632	LEA	0	3							N												
0	3	5/19/87	647	LEA	0	1							N												
0	4	4/24/87	257	LEA	0	1							N												
0	4	4/25/87	392	LEA	0	1							N												
0	4	5/13/87	662	LEA	0	1							N												
0	4	5/19/87	646	LEA	0	1							N												
0	5	4/24/87	210	LEA	0	1							N												
0	5	4/25/87	377	LEA	0	1							N												
0	5	5/01/87	426	LEA	0	1							N												
0	5	5/13/87	626	LEA	0	1							N												
0	5	5/19/87	645	LEA	0	3							N												
0	6	4/24/87	290	LEA	0	1							N												
0	6	4/25/87	349	LEA	0	1							N												
0	6	5/01/87	493	LEA	0	1							N												
0	6	5/13/87	693	LEA	0	1							N												
0	6	5/19/87	643	LEA	0	4							N												
0	7	4/24/87	297	LEA	0	1							N												
0	7	4/25/87	370	LEA	0	3							N												
0	7	5/01/87	483	LEA	0	3							N												
0	7	5/13/87	683	LEA	0	2							N												
0	7	5/19/87	640	LEA	0	1							N												
0	8	4/24/87	285	LEA	0	1							N												
0	8	4/25/87	312	LEA	0	1							N												
0	8	5/01/87	480	LEA	0	1							N												
0	8	5/13/87	680	LEA	0	4							N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987. Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
0	8		5/19/87	634	LEA	0	2						N												
0	9		4/24/87	214	LEA	0	1						N												
0	9		4/25/87	328	LEA	0	1						N												
0	9		5/01/87	468	LEA	0	2						N												
0	9		5/13/87	668	LEA	0	1						N												
0	9		5/19/87	627	LEA	0	1						N												
0	10		4/24/87	296	LEA	0	1						N												
0	10		4/25/87	308	LEA	0	2						N												
0	10		5/01/87	463	LEA	0	2						N												
0	10		5/13/87	663	LEA	0	1						N												
0	10		5/19/87	625	LEA	0	1						N												
0	11		4/24/87	240	LEA	0	3						N												
0	11		4/25/87	378	LEA	0	1						N												
0	11		5/01/87	403	LEA	0	3						N												
0	11		5/13/87	603	LEA	0	1						N												
0	11		5/19/87	623	LEA	0	1						N												
0	12		4/24/87	286	LEA	0	1						N												
0	12		4/25/87	327	LEA	0	1						N												
0	12		5/01/87	411	LEA	0	3						N												
0	12		5/13/87	611	LEA	0	1						N												
0	12		5/19/87	616	LEA	0	1						N												
0	13		4/24/87	207	LEA	0	1						N												
0	13		4/25/87	391	LEA	0	1						N												
0	13		5/01/87	407	LEA	0	1						N												
0	13		5/13/87	694	LEA	0	3						N												
0	13		5/19/87	613	LEA	0	2						N												
0	14		4/24/87	220	LEA	0	1						N												
0	14		4/25/87	399	LEA	0	1						N												
0	14		5/01/87	494	LEA	0	1						N												
0	14		5/13/87	620	LEA	0	4						N												
0	14		5/19/87	612	LEA	0	1						N												
0	15		4/24/87	203	LEA	0	1						N												
0	15		4/25/87	400	LEA	0	1						N												
0	15		5/01/87	420	LEA	0	2						N												
0	15		5/13/87	674	LEA	0	1						N												
0	15		5/19/87	687	LEA	0	1						N												
0	16		4/24/87	275	LEA	0	1						N												

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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987. Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
0	16	4/25/87	361	LEA	0	2							N												
0	16	5/01/87	474	LEA	0	1							N												
0	16	5/13/87	607	LEA	0	2							N												
0	16	5/19/87	686	LEA	0	3							N												
0	17	4/24/87	242	LEA	0	1							N												
0	17	4/25/87	374	LEA	0	2							N												
0	17	5/01/87	431	LEA	0	1							N												
0	17	5/13/87	631	LEA	0	1							N												
0	17	5/19/87	682	LEA	0	1							N												
0	18	4/24/87	231	LEA	0	1							N												
0	18	4/25/87	309	LEA	0	0							N												
0	18	5/01/87	406	LEA	0	1							N												
0	18	5/13/87	606	LEA	0	3							N												
0	18	5/19/87	681	LEA	0	1							N												
0	19	4/24/87	223	LEA	0	1							N												
0	19	4/25/87	384	LEA	0	1							N												
0	19	5/01/87	442	LEA	0	1							N												
0	19	5/13/87	642	LEA	0	4							N												
0	19	5/19/87	679	LEA	1	1	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
0	20	4/24/87	276	LEA	0	1							N												
0	20	4/25/87	332	LEA	0	1							N												
0	20	5/01/87	466	LEA	0	1							N												
0	20	5/13/87	666	LEA	0	2							N												
0	20	5/19/87	671	LEA	0	1							N												
0	21	4/24/87	247	LEA	0	1							N												
0	21	4/25/87	382	LEA	0	1							N												
0	21	5/01/87	478	LEA	0	1							N												
0	21	5/13/87	678	LEA	0	0							N												
0	21	5/19/87	664	LEA	0	3							N												
0	22	4/24/87	258	LEA	0	1							N												
0	22	4/25/87	324	LEA	0	1							N												
0	22	5/01/87	461	LEA	0	1							N												
0	22	5/13/87	661	LEA	0	1							N												
0	22	5/19/87	660	LEA	0	1							N												
0	23	5/13/87	669	LEA	0	1							N												
0	23	5/19/87	659	LEA	0	2							N												
0	24	4/24/87	238	LEA	0	1							N												
0	24	4/25/87	353	LEA	0	1							N												

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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
0	24	5/01/87	444	LEA	0	1							N												
0	24	5/13/87	644	LEA	0	1							N												
0	24	5/19/87	655	LEA	0	1							N												
0	25	5/13/87	617	LEA	0	1							N												
0	25	5/19/87	700	LEA	0	1							N												
0	26	4/24/87	248	LEA	0	1							N												
0	26	4/25/87	372	LEA	0	1							N												
0	26	5/01/87	498	LEA	0	1							N												
0	26	5/13/87	698	LEA	0	1							N												
0	26	5/19/87	696	LEA	0	2							N												
0	27	4/24/87	241	LEA	0	1							N												
0	27	4/25/87	366	LEA	0	2							N												
0	27	5/01/87	467	LEA	0	2							N												
0	27	5/13/87	667	LEA	0	1							N												
0	27	5/19/87	695	LEA	0	1							N												
0	28	4/24/87	202	LEA	0	1							N												
0	28	4/25/87	362	LEA	0	1							N												
0	28	5/01/87	439	LEA	0	2							N												
0	28	5/13/87	639	LEA	0	1							N												
0	28	5/19/87	692	LEA	0	1							N												
0	29	4/24/87	229	LEA	0	1							N												
0	29	4/25/87	373	LEA	0	1							N												
0	29	5/01/87	414	LEA	0	2							N												
0	29	5/13/87	614	LEA	0	1							N												
0	29	5/19/87	658	LEA	0	3							N												
0	30	4/24/87	293	LEA	0	1							N												
0	30	4/25/87	321	LEA	0	1							N												
0	30	5/01/87	415	LEA	0	1							N												
0	30	5/13/87	615	LEA	0	4							N												
0	30	5/19/87	701	LEA	0	2							N												
0	31	4/24/87	287	LEA	0	1							N												
0	31	4/25/87	306	LEA	0	1							N												
0	31	5/01/87	472	LEA	0	1							N												
0	31	5/13/87	672	LEA	0	4							N												
0	31	5/19/87	702	LEA	0	1							N												
0	32	4/24/87	270	LEA	0	1							N												
0	32	4/25/87	347	LEA	0	1							N												
0	32	5/01/87	424	LEA	0	1							N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	REF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
0	32	5/13/87	624	LEA	0	2							N												
0	32	5/19/87	703	LEA	0	1							N												
0	33	4/24/87	295	LEA	0	1							N												
0	33	4/25/87	358	LEA	0	1							N												
0	33	5/01/87	436	LEA	0	0							N												
0	33	5/13/87	636	LEA	0	4							N												
0	33	5/19/87	704	LEA	0	1							N												
0	34	4/24/87	233	LEA	0	1							N												
0	34	4/25/87	315	LEA	0	1							N												
0	34	5/01/87	488	LEA	0	1							N												
0	34	5/13/87	688	LEA	0	2							N												
0	34	5/19/87	705	LEA	0	3							N												
0	35	4/24/87	254	LEA	0	1							N												
0	35	4/25/87	338	LEA	0	2							N												
0	35	5/01/87	452	LEA	0	2							N												
0	35	5/13/87	652	LEA	0	1							N												
0	35	5/19/87	706	LEA	0	1							N												
0	36	4/24/87	216	LEA	0	1							N												
0	36	4/25/87	394	LEA	0	2							N												
0	36	5/01/87	449	LEA	0	1							N												
0	36	5/13/87	649	LEA	0	1							N												
0	36	5/19/87	707	LEA	0	2							N												
0	37	4/24/87	289	LEA	0	1							N												
0	37	4/25/87	348	LEA	0	2							N												
0	37	5/01/87	457	LEA	0	1							N												
0	37	5/13/87	657	LEA	0	2							N												
0	37	5/19/87	708	LEA	0	1							N												
0	38	4/24/87	265	LEA	0	1							N												
0	38	4/25/87	341	LEA	0	1							N												
0	38	5/01/87	470	LEA	0	1							N												
0	38	5/13/87	670	LEA	0	1							N												
0	38	5/19/87	709	LEA	0	1							N												
0	39	4/24/87	201	LEA	0	1							N												
0	39	4/25/87	302	LEA	0	2							N												
0	39	5/01/87	405	LEA	0	1							N												
0	39	5/13/87	605	LEA	0	1							N												
0	39	5/19/87	710	LEA	0	1							N												
0	40	4/24/87	211	LEA	0	1							N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
O	40	4/25/87	329	LEA	0	1							N												
O	40	5/01/87	422	LEA	0	1							N												
O	40	5/13/87	622	LEA	0	2							N												
O	40	5/19/87	711	LEA	0	2							N												
P		4/10/87	9	LEA	0	1							N												
P		4/10/87	18	LEA	0	4							N												
P		4/10/87	25	LEA	0	1							N												
P		4/10/87	26	LEA	0	1							N												
P		4/10/87	37	LEA	0	1							N												
P		4/10/87	46	LEA	0	1							N												
P		4/10/87	51	LEA	0	1							N												
P		4/10/87	89	LEA	0	1							N												
P		4/24/87	209	LEA	0	2							N												
P		4/24/87	221	LEA	0	2							N												
P		4/24/87	224	LEA	0	2							N												
P		4/24/87	232	LEA	0	4							N												
P		4/24/87	253	LEA	0	2							N												
P		4/24/87	262	LEA	0	2							N												
P		4/24/87	266	LEA	0	1							N												
P		4/24/87	272	LEA	0	2							N												
P		4/24/87	273	LEA	0	2							N												
P		4/24/87	274	LEA	0	2							N												
P		4/24/87	282	LEA	0	2							N												
P		4/24/87	284	LEA	0	2							N												
P		5/01/87	401	LEA	0	2							N												
P		5/01/87	404	LEA	0	0							N												
P		5/01/87	418	LEA	0	2							N												
P		5/01/87	430	LEA	0	2							N												
P		5/01/87	435	LEA	0	1							N												
P		5/01/87	437	LEA	0	1							N												
P		5/01/87	438	LEA	0	2							N												
P		5/01/87	456	LEA	0	1							N												
P		5/01/87	465	LEA	0	2							N												
P		5/01/87	476	LEA	0	1							N												
P		5/01/87	484	LEA	0	1							N												
P		5/01/87	497	LEA	0	3							N												
P		5/08/87	501	LEA	0	2							N												
P		5/08/87	504	LEA	0	1							N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS[d] A	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
P			5/08/87	518	LEA	0	2						N												
P			5/08/87	530	LEA	0	2						N												
P			5/08/87	535	LEA	0	2						N												
P			5/08/87	537	LEA	0	0						N												
P			5/08/87	538	LEA	0	1						N												
P			5/08/87	556	LEA	0	2						N												
P			5/08/87	565	LEA	0	1						N												
P			5/08/87	576	LEA	0	1						N												
P			5/08/87	584	LEA	0	2						N												
P			5/08/87	597	LEA	0	1						N												
P			5/13/87	604	LEA	0	1						N												
P			5/13/87	610	LEA	0	3						N												
P			5/13/87	618	LEA	0	4						N												
P			5/13/87	630	LEA	0	1						N												
P			5/13/87	635	LEA	0	4						N												
P			5/13/87	637	LEA	0	2						N												
P			5/13/87	638	LEA	0	2						N												
P			5/13/87	656	LEA	0	1						N												
P			5/13/87	665	LEA	0	1						N												
P			5/13/87	676	LEA	0	1						N												
P			5/13/87	684	LEA	0	1						N												
P			5/13/87	697	LEA	0	1						N												
P			5/19/87	716	LEA	0	2						N												
P			5/19/87	718	LEA	0	3						N												
P			5/19/87	730	LEA	0	3						N												
P			5/19/87	735	LEA	0	2						N												
P			5/19/87	737	LEA	0	1						N												
P			5/19/87	738	LEA	0	1						N												
P			5/19/87	756	LEA	0	1						N												
P			5/19/87	758	LEA	0	2						N												
P			5/19/87	765	LEA	0	1						N												
P			5/19/87	776	LEA	0	2						N												
P			5/19/87	784	LEA	0	1						N												
P			5/19/87	797	LEA	0	1						N												
P			5/26/87	816	LEA	0	2						N												
P			5/26/87	818	LEA	0	1						N												
P			5/26/87	830	LEA	0	1						N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	REP (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
P			5/26/87	835	LEA	0	1						N												
P			5/26/87	837	LEA	0	1						N												
P			5/26/87	838	LEA	0	1						N												
P			5/26/87	856	LEA	0	2						N												
P			5/26/87	858	LEA	0	3						N												
P			5/26/87	865	LEA	0	2						N												
P			5/26/87	876	LEA	0	1						N												
P			5/26/87	884	LEA	0	1						N												
P			5/26/87	897	LEA	0	2						N												
P			6/02/87	909	LEA	0	1						N												
P			6/02/87	914	LEA	0	1						N												
P			6/02/87	918	LEA	0	1						N												
P			6/02/87	925	LEA	0	2						N												
P			6/02/87	926	LEA	0	1						N												
P			6/02/87	937	LEA	0	1						N												
P			6/02/87	944	LEA	0	1						N												
P			6/02/87	946	LEA	0	1						N												
P			6/02/87	951	LEA	0	1						N												
P			6/02/87	863	LEA	0	1						N												
P			6/02/87	983	LEA	0	1						N												
P			6/02/87	989	LEA	0	1						N												
P			6/09/87	1009	LEA	0	1						N												
P			6/09/87	1014	LEA	0	1						N												
P			6/09/87	1018	LEA	0	1						N												
P			6/09/87	1025	LEA	0	0						N												
P			6/09/87	1026	LEA	0	0						N												
P			6/09/87	1037	LEA	0	2						N												
P			6/09/87	1044	LEA	0	0						N												
P			6/09/87	1046	LEA	0	2						N												
P			6/09/87	1051	LEA	0	2						N												
P			6/09/87	1063	LEA	0	0						N												
P			6/09/87	1083	LEA	0	0						N												
P			6/09/87	1089	LEA	0	2						N												
S			4/10/87	24	LEA	0	1						N												
S			4/10/87	40	LEA	0	2						N												
S			4/10/87	47	LEA	0	2						N												
S			4/10/87	49	LEA	0	2						N												
S			4/10/87	50	FLO	0	3						N												

APPENDIX II
Page 14
Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987. Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
S			4/10/87	52	LEA	0	2						N												
S			4/10/87	58	LEA	0	4						N												
S			4/10/87	78	FLO	0	2						N												
S			4/10/87	80	LEA	0	3						N												
S			4/10/87	99	LEA	0	2						N												
S			4/24/87	204	LEA	0	4						N												
S			4/24/87	208	LEA	0	2						N												
S			4/24/87	212	LEA	0	3						N												
S			4/24/87	225	FLO	0	4						N												
S			4/24/87	228	FLO	0	4						N												
S			4/24/87	236	LEA	0	4						N												
S			4/24/87	249	LEA	0	4						N												
S			4/24/87	252	LEA	0	3						N												
S			4/24/87	277	LEA	0	4						N												
S			4/24/87	292	LEA	0	4						N												
S			5/01/87	408	LEA	0	3						N												
S			5/01/87	428	LEA	0	2						N												
S			5/01/87	453	LEA	0	4						N												
S			5/02/87	454	FLO	0	4						N												
S			5/01/87	473	LEA	0	3						N												
S			5/01/87	475	LEA	0	2						N												
S			5/01/87	477	LEA	0	3						N												
S			5/01/87	489	FLO	0	3						N												
S			5/01/87	490	LEA	0	3						N												
S			5/01/87	491	LEA	0	2						N												
S			5/08/87	508	LEA	0	1						N												
S			5/08/87	528	LEA	0	3						N												
S			5/08/87	553	LEA	0	3						N												
S			5/08/87	554	FLO	0	4						N												
S			5/08/87	573	LEA	0	0						N												
S			5/08/87	575	LEA	0	2						N												
S			5/08/87	577	LEA	0	2						N												
S			5/08/87	589	FLO	0	4						N												
S			5/08/87	590	LEA	0	4						N												
S			5/08/87	591	LEA	0	3						N												
S			5/13/87	608	LEA	0	2						N												
S			5/13/87	628	LEA	0	3						N												
S			5/13/87	653	LEA	0	2						N												

APPENDIX II
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Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
S			5/13/87	654	FLO	0	4						N												
S			5/13/87	673	LEA	0	3						N												
S			5/13/87	675	LEA	0	4						N												
S			5/13/87	677	LEA	0	4						N												
S			5/13/87	689	FLO	0	2						N												
S			5/13/87	690	LEA	0	3						N												
S			5/13/87	691	LEA	0	4						N												
S			5/19/87	728	LEA	0	1						N												
S			5/19/87	743	LEA	0	2						N												
S			5/19/87	753	LEA	0	1						N												
S			5/19/87	754	FLO	0	3						N												
S			5/19/87	773	LEA	0	1						N												
S			5/19/87	775	LEA	0	1						N												
S			5/19/87	777	LEA	0	1						N												
S			5/19/87	789	FLO	0	3						N												
S			5/19/87	790	LEA	0	1						N												
S			5/19/87	791	LEA	0	2						N												
S			5/26/87	828	LEA	0	3						N												
S			5/26/87	843	LEA	0	1						N												
S			5/26/87	853	LEA	0	2						N												
S			5/26/87	854	FLO	0	3						N												
S			5/26/87	873	LEA	0	3						N												
S			5/26/87	875	LEA	0	1						N												
S			5/26/87	877	LEA	0	3						N												
S			5/26/87	889	LEA	0	2						N												
S			5/26/87	890	LEA	0	4						N												
S			5/26/87	891	LEA	0	4						N												
S			6/02/87	924	LEA	0	1						N												
S			6/02/87	940	LEA	0	1						N												
S			6/02/87	947	LEA	0	1						N												
S			6/02/87	949	LEA	0	4						N												
S			6/02/87	950	LEA	0	1						N												
S			6/02/87	952	LEA	0	1						N												
S			6/02/87	958	LEA	0	1						N												
S			6/02/87	978	LEA	0	1						N												
S			6/02/87	980	LEA	0	1						N												
S			6/02/87	999	LEA	0	2						N												
S			6/09/87	1024	LEA	0							N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A[d]	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
S			6/09/87	1040	LEA	0	2						N												
S			6/09/87	1047	LEA	0	1						N												
S			6/09/87	1049	LEA	0	1						N												
S			6/09/87	1050	LEA	0	1						N												
S			6/09/87	1052	LEA	0	0						N												
S			6/09/87	1058	LEA	0	1						N												
S			6/09/87	1078	LEA	0	4						N												
S			6/09/87	1080	LEA	0	0						N												
S			6/09/87	1099	LEA	0	1						N												
T			4/10/87	21	LEA	0	2						N												
T			4/24/87	206	LEA	0	4						N												
T			5/01/87	441	LEA	0	3						N												
T			5/08/87	541	LEA	0	2						N												
T			5/13/87	641	LEA	0	4						N												
T			5/19/87	741	LEA	0	2						N												
T			5/26/87	841	LEA	0	2						N												
T			6/02/87	921	LEA	0	2						N												
T			6/09/87	1021	LEA	0	1						N												
W			4/10/87	14	LEA	0	2						N												
W			4/10/87	15	LEA	0	4						N												
W			4/10/87	44	LEA	0	1						N												
W			4/10/87	53	LEA	0	1						N												
W			4/10/87	63	LEA	0	1						N												
W			4/10/87	83	LEA	0	1						N												
W			4/10/87	84	LEA	0	2						N												
W			4/10/87	94	LEA	0	2						N												
W			4/24/87	261	LEA	0	3						N												
W			4/24/87	268	LEA	0	3						N												
W			4/24/87	278	LEA	0	0						N												
W			4/24/87	300	LEA	0	3						N												
W			5/01/87	409	LEA	0	4						N												
W			5/01/87	410	LEA	0	1						N												
W			5/01/87	433	LEA	0	2						N												
W			5/01/87	485	LEA	0	4						N												
W			5/08/87	509	LEA	0	3						N												
W			5/08/87	519	LEA	0	3						N												
W			5/08/87	533	LEA	0	3						N												
W			5/08/87	585	FLO	0	4						N												

Diagnostic test results, final diagnoses and abundance of fluorescent bacteria from field samples collected in the spring, 1987, Frostban Study.

AREA (a)	PLOT	FLAT	DATE	SAMPLE	TYPE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A (d)	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
W			5/13/87	609	LEA	0	3						N												
W			5/13/87	619	LEA	0	1						N												
W			5/13/87	633	LEA	0	4						N												
W			5/13/87	685	FLO	0	3						N												
W			5/19/87	719	LEA	0	1						N												
W			5/19/87	733	LEA	0	4						N												
W			5/19/87	746	LEA	0	3						N												
W			5/19/87	785	FLO	0	4						N												
W			5/26/87	819	LEA	0	1						N												
W			5/26/87	833	LEA	0	1						N												
W			5/26/87	846	LEA	0	4						N												
W			5/26/87	746	LEA	0	4						N												
W			6/02/87	915	FLO	0	4						N												
W			6/02/87	953	LEA	0	1						N												
W			5/02/87	984	LEA	0	2						N												
W			6/02/87	994	LEA	0	1						N												
W			6/09/87	1015	FLO	0	4						N												
W			6/09/87	1053	LEA	0	4						N												
W			6/09/87	1084	LEA	0	2						N												
W			6/09/87	1094	LEA	0	1						N												

a. A= Apricot
F= Strawberry test site
P= Pear
T= Tomato
C= Corn
O= Perimeter oat flats
S= Off-site strawberry
W= Off-site weeds

b. Rif= Abundance of fluorescent bacteria on kings b medium amended with rifampicin.

c. Cyclo= Abundance of fluorescent bacteria on kings b medium. The following scheme was used for abundance determination for culture plates: 0= no colonies present; 1= 1 to 10 colonies present; 2= 11 to 100 colonies; 3= 101 to 1000 colonies; 4= too numerous to count.

d. Final diagnosis results:
F= Genetically engineered P. fluorescens
S= Genetically engineered P. syringae
B= Both genetically engineered Pseudomonas species
N= No genetically engineered bacteria

Diagnostic tests: Fluor= fluorescence test; Oxidase= oxidase test; Arginine= arginine test; Ice= ice nucleation test; Probe= gene probe analysis; Diagnosis= final diagnosis by CDFA lab. Letter after diagnostic test refers to isolate tested.

APPENDIX III

DIAGNOSTIC TEST RESULTS AND FINAL DIAGNOSES FOR QUALITY CONTROL SAMPLES

APPENDIX III

Diagnostic test results and final diagnoses for spiked foliage submitted as quality control samples for the spring, 1987, Frostban® Monitoring Study.

QCIC (a)	DATE	RIF (b)	CVCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
B	4/10/87	3	4	+	+	+	-	N	F	+	+	+	-	N	F	+	-	-	-	S	S
B	4/10/87	3	4	+	-	-	-	F	S	+	+	+	-	N	F	+	-	-	-	N	S
B	4/24/98	2	3	+	-	-	-	S	S	+	+	+	-	F	F	+	-	-	-	S	S
B	4/24/87	2	3	+	-	-	-	S	S	+	+	+	-	N	F	+	-	-	-	F	S
B	5/01/87	3	4	+	+	+	-	N	F	+	+	+	-	F	F	+	-	-	-	F	S
B	5/01/87	3	4	+	+	+	-	N	F	+	-	-	-	S	S	+	-	-	-	S	F
B	5/08/87	1	3	+	-	-	-	S	S	+	+	+	-	F	F	+	-	-	-	S	S
B	5/08/87	2	3	+	+	+	-	S	F	+	-	-	-	S	S	+	-	-	-	S	S
B	5/13/87	3	4	+	-	-	-	N	S	+	+	+	-	F	F	+	+	+	-	F	F
B	5/13/87	4	4	+	+	+	-		F	+	-	-	-	N	S	+	-	-	-	F	S
B	5/19/87	3	3	+	-	-	-	S	S	+	+	+	-	N	F	+	-	-	-	S	S
B	5/19/87	4	4	+	-	-	-	S	S	+	+	+	-	F	F	+	-	-	-	S	S
B	5/26/87	3	3	+	-	-	-	S	S	+	+	+	-	F	F	+	+	+	-	F	F
B	5/26/87	3	4	+	-	-	-	S	S	+	+	+	-	N	F	+	-	-	-	S	S
B	6/02/87	2	4	+	+	+	-	F	F	+	-	-	-	S	S	+	+	+	-	F	F
B	6/02/87	3	4	+	-	-	-	S	S	+	+	+	-	F	F	+	-	-	-	S	S
B	6/09/87	4	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
B	6/09/87	4	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	4/10/87	3	3	+	+	+	-	N	F	+	+	+	-	N	F	+	+	+	-	N	F
F	4/10/87	3	3	+	+	+	-	N	F	+	+	+	-	N	F	+	+	+	-	N	F
F	4/24/87	2	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	4/24/87	2	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	5/01/87	3	3	+	+	+	-	S	F	+	+	+	-	S	F	+	+	+	-	S	F
F	5/01/87	3	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	5/08/87	3	3	+	+	+	-	S	F	+	+	+	-	N	F	+	+	+	-		F
F	5/08/87	3	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	5/13/87	3	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	5/13/87	3	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	5/19/87	3	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	N	F
F	5/19/87	4	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	5/26/87	3	3	+	+	+	-	N	F	+	-	-	-	S	S	+	-	-	-	S	S
F	5/26/87	4	4	+	+	+	-	F	F	+	-	-	-	S	S	+	+	+	-	F	F
F	6/02/87	2	3	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6/02/87	2	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6/09/87	4	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
F	6/09/87	4	4	+	+	+	-	F	F	+	+	+	-	F	F	+	+	+	-	F	F
S	4/10/87	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S

QCIC (a)	DATE	RIF (b)	CYCLO (c)	FLUOR A	OXIDASE A	ARGININE A	ICE A	PROBE A	DIAGNOSIS A	FLUOR B	OXIDASE B	ARGININE B	ICE B	PROBE B	DIAGNOSIS B	FLUOR C	OXIDASE C	ARGININE C	ICE C	PROBE C	DIAGNOSIS C
S	4/10/87	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	4/24/87	2	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	4/24/87	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/01/87	3	3	+	-	-	-	N	S	+	-	-	-	N	S	+	-	-	-	S	S
S	5/01/87	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/08/87	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/08/87	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/13/87	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/13/87	3	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/19/87	4	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/19/87	4	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	5/26/87	2	4	+	-	-	-	S	S	+	-	-	-	N	S	+	-	-	-	N	S
S	5/26/87	3	3	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	6/02/87	0	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	6/02/87	2	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	6/09/87	4	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S
S	6/09/87	4	4	+	-	-	-	S	S	+	-	-	-	S	S	+	-	-	-	S	S

a. Identity of bacteria applied to foliage or submitted as control cultures.
F= Genetically engineered F. fluorescens
S= Genetically engineered P. syringae
B= Both genetically engineered Pseudomonas species
N= No genetically engineered bacteria

b. RIF= Abundance of fluorescent bacteria on Kings B medium amended with rifampicin.

c. Cyclo= Abundance of fluorescent bacteria in Kings B medium. The following rating scheme was used for abundance determination for culture plates:
0= no colonies present; 1= 1-10 colonies present; 2= 11-100 colonies; 3= 100-1000 colonies; 4= greater than 1000 colonies. Fluor= fluorescence test: Oxidase= oxidase test; Arginine= arginine dihydrolase test;
Ice= ice nucleation test; Probe= gene probe analysis results; Diagnosis= final diagnosis by CDFA Plant Pathology Lab.
+= positive; -= negative; ?= uncertain. Number after diagnostic test refers to isolate tested.

APPENDIX IV

GENETICALLY ENGINEERED BACTERIA APPLIED AND RECOVERED ON SITE

APPENDIX IV

Genetically engineered Pseudomonas species applied and recovered from on-site strawberry plants during the sampling period. Dates of first and second Frostban® applications were April 24, 1987 and May 12, 1987, respectively.

Strawberry Plot	Sample Type	Date	Treatment Type[a]	Pseudomonas spp. recovered by CDFA[b]
1	FLO	4/10/87	B	N
1	LEA	4/10/87	B	N
1	LEA	4/24/87	F	F
1	LEA	5/01/87	F	F
1	LEA	5/08/87	F	F
1	LEA	5/13/87	F	F
1	LEA	5/19/87	F	F
1	LEA	5/26/87	F	F
1	LEA	6/02/87	F	N
2	FLO	4/10/87	B	N
2	LEA	4/10/87	B	N
2	LEA	4/24/87	S	S
2	LEA	5/01/87	S	S
2	LEA	5/08/87	S	S
2	LEA	5/13/87	S	S
2	LEA	5/19/87	S	S
2	LEA	5/26/87	S	S
2	LEA	6/02/87	S	N
3	FLO	4/10/87	B	N
3	LEA	4/10/87	B	N
3	LEA	4/24/87	C	N
3	LEA	5/01/87	C	N
3	LEA	5/08/87	C	N
3	LEA	5/13/87	C	N
3	LEA	5/19/87	C	N
3	LEA	5/26/87	C	N
3	LEA	6/02/87	C	N
4	FLO	4/10/87	B	N
4	LEA	4/10/87	B	N
4	LEA	4/24/87	K	N
4	LEA	5/01/87	K	N
4	LEA	5/08/87	K	N
4	LEA	5/13/87	K	N
4	LEA	5/19/87	K	N
4	LEA	5/26/87	K	N
4	LEA	6/02/87	K	N
5	FLO	4/10/87	B	N
5	LEA	4/10/87	B	N
5	LEA	4/24/87	C	N
5	LEA	5/01/87	C	N
5	LEA	5/08/87	C	N
5	LEA	5/13/87	C	N
5	LEA	5/19/87	C	N
5	LEA	5/26/87	C	N
5	LEA	6/02/87	C	N

Strawberry Plot	Sample Type	Date	Treatment Type[a]	Pseudomonas spp. recovered by CDFA[b]
6	FLO	4/10/87	B	N
6	LEA	4/10/87	B	N
6	LEA	4/24/87	F	F
6	LEA	5/01/87	F	F
6	LEA	5/08/87	F	F
6	LEA	5/13/87	F	F
6	LEA	5/19/87	F	N
6	LEA	5/26/87	F	F
6	LEA	6/02/87	F	N
7	FLO	4/10/87	B	N
7	LEA	4/10/87	B	N
7	LEA	4/24/87	K	N
7	LEA	5/01/87	K	N
7	LEA	5/08/87	K	N
7	LEA	5/13/87	K	N
7	LEA	5/19/87	K	N
7	LEA	5/26/87	K	N
7	LEA	6/02/87	K	N
8	FLO	4/10/87	B	N
8	LEA	4/10/87	B	N
8	LEA	4/24/87	S	S
8	LEA	5/01/87	S	N
8	LEA	5/08/87	S	S
8	LEA	5/13/87	S	S
8	LEA	5/19/87	S	S
8	LEA	5/26/87	S	S
8	LEA	6/02/87	S	N
9	FLO	4/10/87	B	N
9	LEA	4/10/87	B	N
9	LEA	4/24/87	S	S
9	LEA	5/01/87	S	S
9	LEA	5/13/87	S	S
9	LEA	5/13/87	S	S
9	LEA	5/19/87	S	S
9	LEA	5/26/87	S	S
9	LEA	6/02/87	S	N
10	FLO	4/10/87	B	N
10	LEA	4/10/87	B	N
10	LEA	4/24/87	K	N
10	LEA	5/01/87	K	N
10	LEA	5/08/87	K	N
10	LEA	5/13/87	K	N
10	LEA	5/19/87	K	N
10	LEA	5/26/87	K	N
10	LEA	6/02/87	K	N
11	FLO	4/10/87	B	N
11	LEA	4/10/87	B	N
11	LEA	4/24/87	F	F
11	LEA	5/01/87	F	F

Strawberry Plot	Sample Type	Date	Treatment Type[a]	Pseudomonas spp. recovered by CDFA[b]
11	LEA	5/08/87	F	F
11	LEA	5/13/87	F	N
11	LEA	5/19/87	F	F
11	LEA	5/26/87	F	F
11	LEA	6/02/87	F	N
12	FLO	4/10/87	C	N
12	LEA	4/10/87	C	N
12	LEA	4/24/87	C	N
12	LEA	5/01/87	C	N
12	LEA	5/08/87	C	N
12	LEA	5/13/87	C	N
12	LEA	5/19/87	C	N
12	LEA	5/26/87	C	N
12	LEA	6/02/87	C	N
13	FLO	4/10/87	B	N
13	LEA	4/10/87	B	N
13	LEA	4/24/87	K	N
13	LEA	5/01/87	K	N
13	LEA	5/08/87	K	N
13	LEA	5/13/87	K	N
13	LEA	5/19/87	K	N
13	LEA	5/26/87	K	N
13	LEA	6/02/87	K	N
14	FLO	4/10/87	B	N
14	LEA	4/10/87	B	N
14	LEA	4/24/87	C	N
14	LEA	5/01/87	C	N
14	LEA	5/08/87	C	N
14	LEA	5/13/87	C	N
14	LEA	5/19/87	C	N
14	LEA	5/26/87	C	N
14	LEA	6/02/87	C	N
15	FLO	4/10/87	B	N
15	LEA	4/10/87	B	N
15	LEA	4/24/87	S	S
15	LEA	5/01/87	S	S
15	LEA	5/08/87	S	S
15	LEA	5/13/87	S	S
15	LEA	5/19/87	S	S
15	LEA	5/26/87	S	S
15	LEA	6/02/87	S	S
16	FLO	4/10/87	B	N
16	LEA	4/10/87	B	N
16	LEA	4/24/87	F	S
16	LEA	5/01/87	F	F
16	LEA	5/08/87	F	F
16	LEA	5/13/87	F	F
16	LEA	5/19/87	F	N
16	LEA	5/26/87	F	N

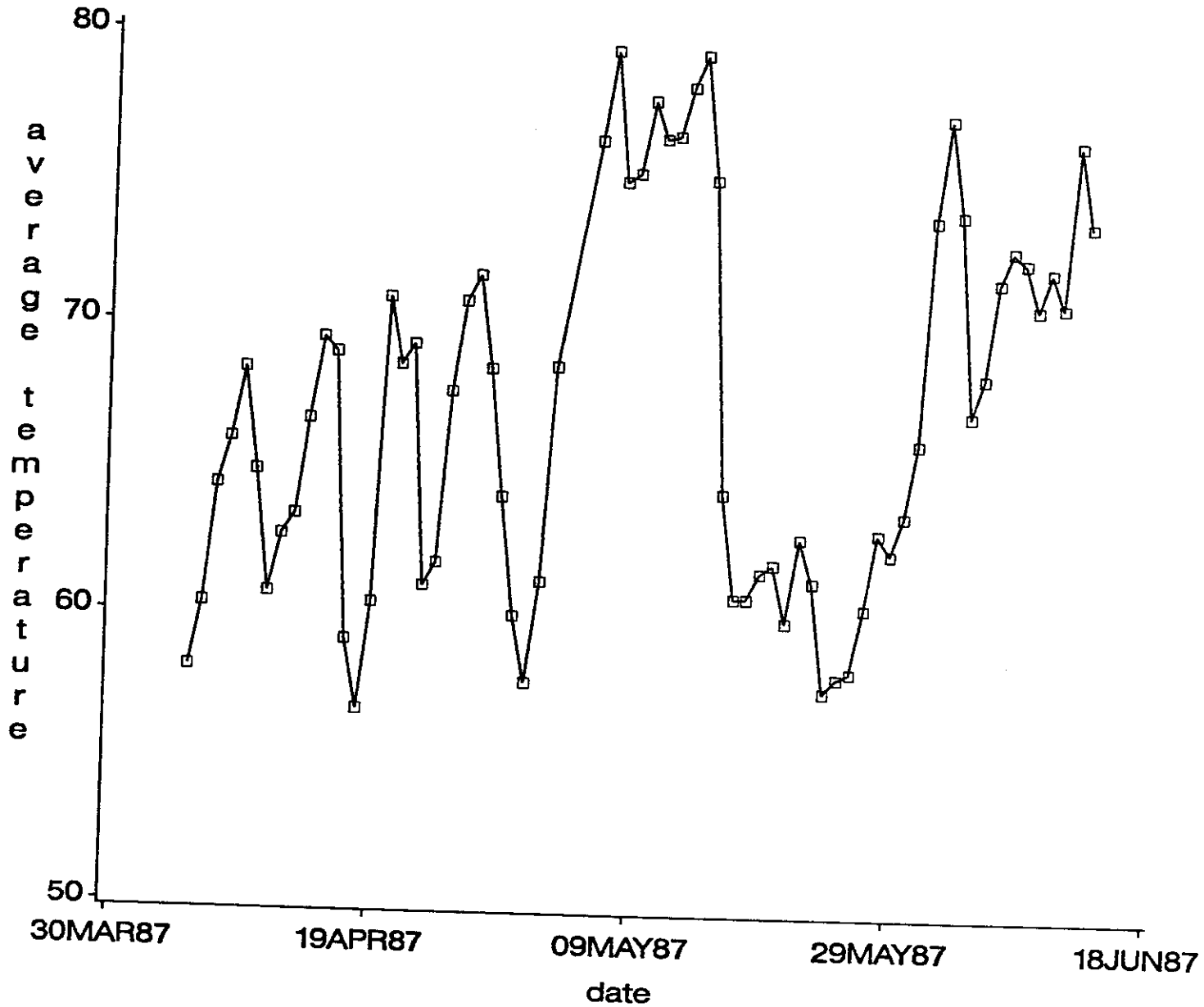
Strawberry Plot	Sample Type	Date	Treatment Type[a]	Pseudomonas spp. recovered by CDFA[b]
16	LEA	6/02/87	F	N

a. B= Background
S= Genetically engineered P. syringae applied.
F= Genetically engineered P. fluorescens applied.
C= Control; phosphate buffer only applied.
K= Bactericide applied.

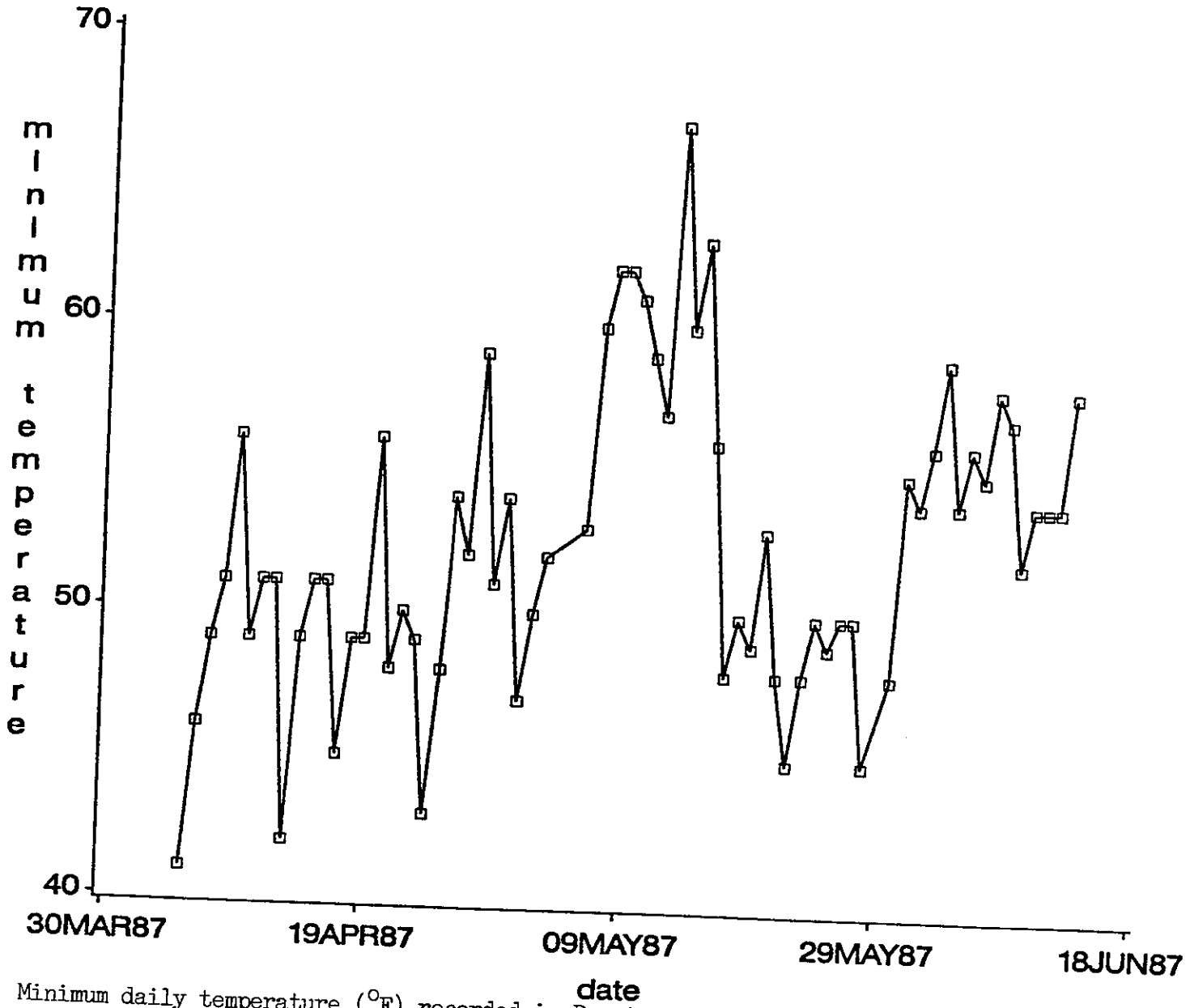
b. S= Genetically engineered P. syringae. F= Genetically engineered P. fluorescens. N= No genetically engineered bacteria.

APPENDIX V

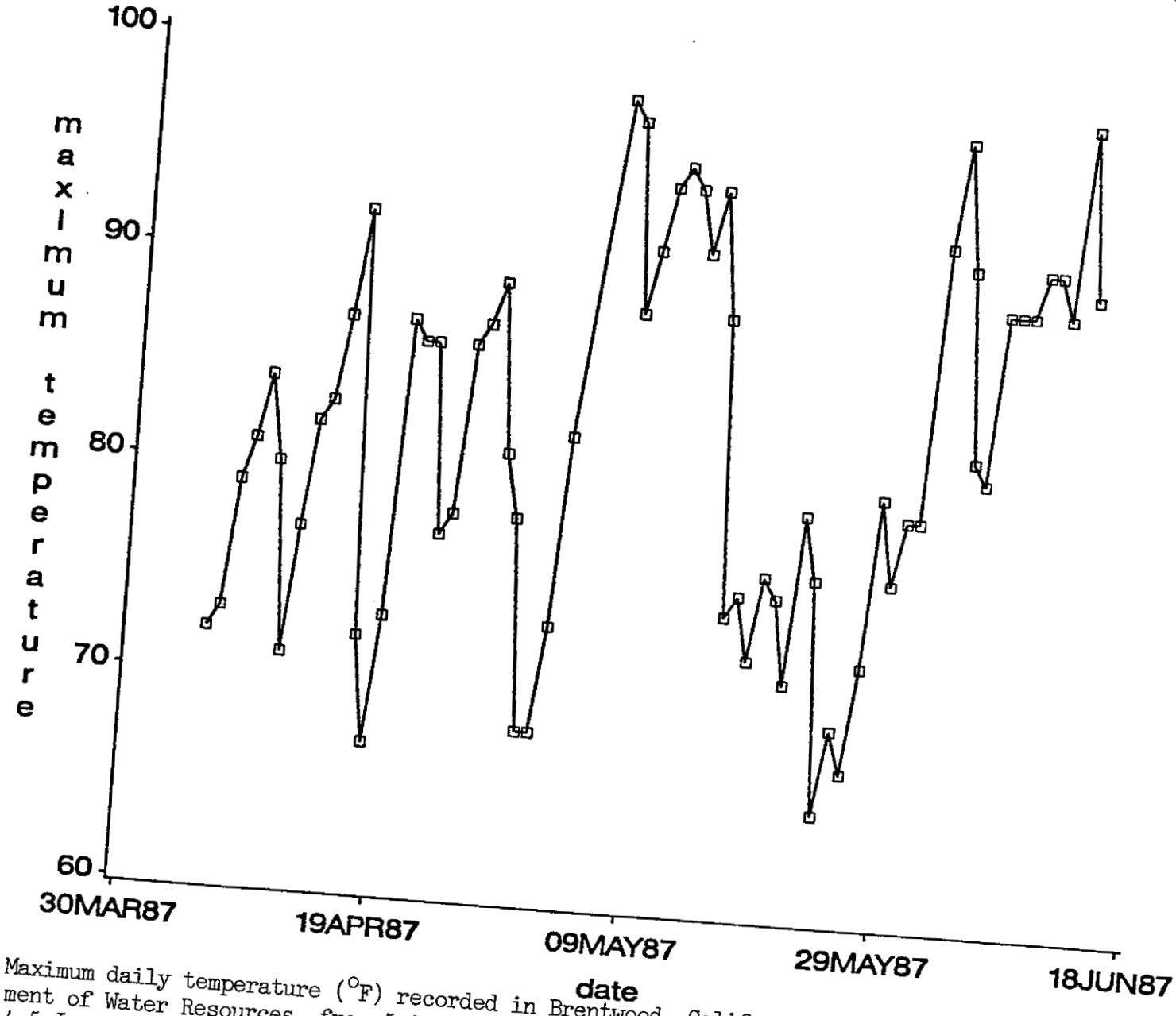
WEATHER DATA FOR BRENTWOOD



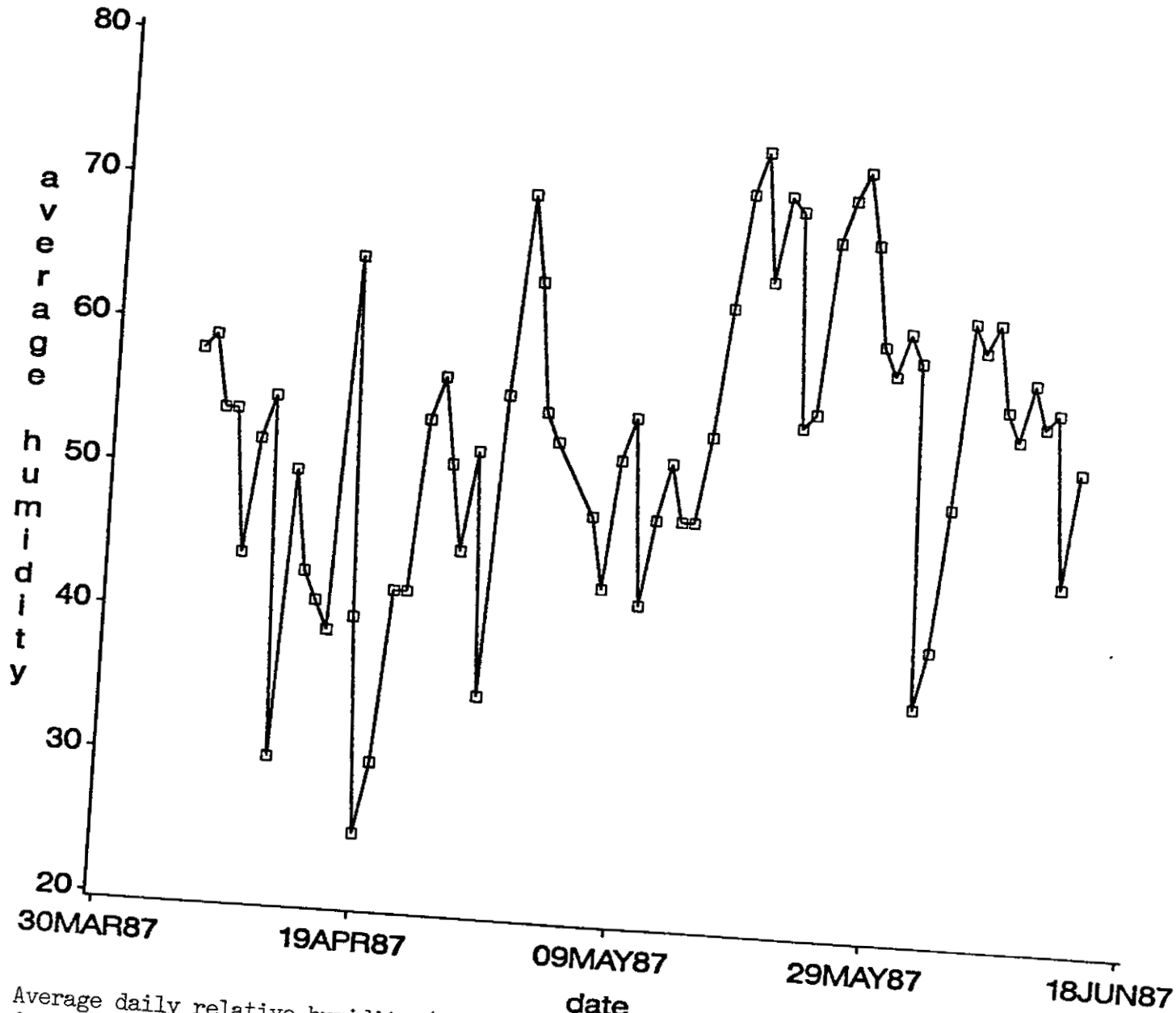
Average daily temperature (^{o}F) recorded in Brentwood, California, by the California Department of Water Resources, from 5 April, 1987 to 13 June, 1987. No data are available for 4-5 June.



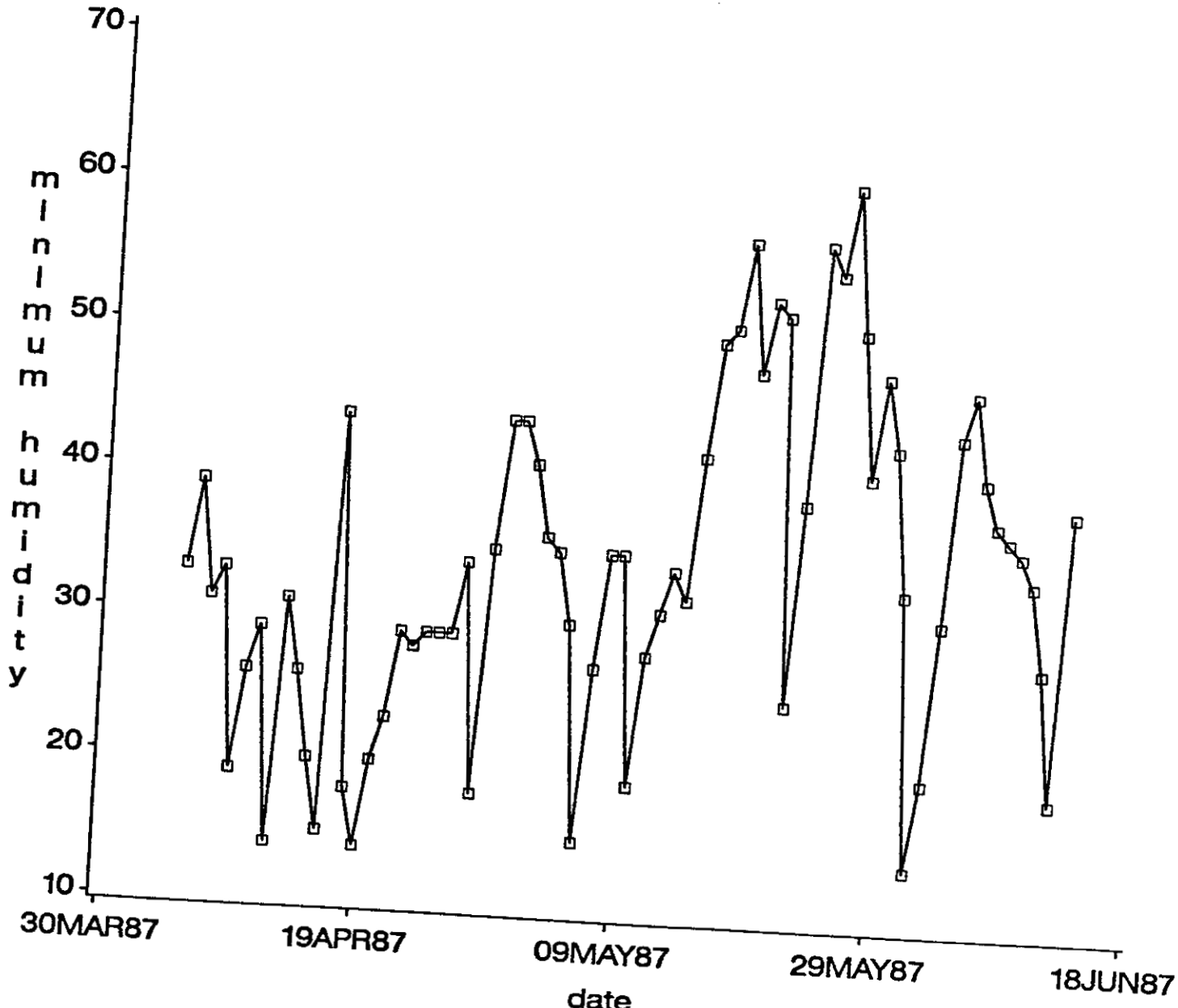
Minimum daily temperature (^{o}F) recorded in Brentwood, California, by the California Department of Water Resources, from 5 April, 1987 to 13 June, 1987. No data are available for 4, 5, and 29 June.



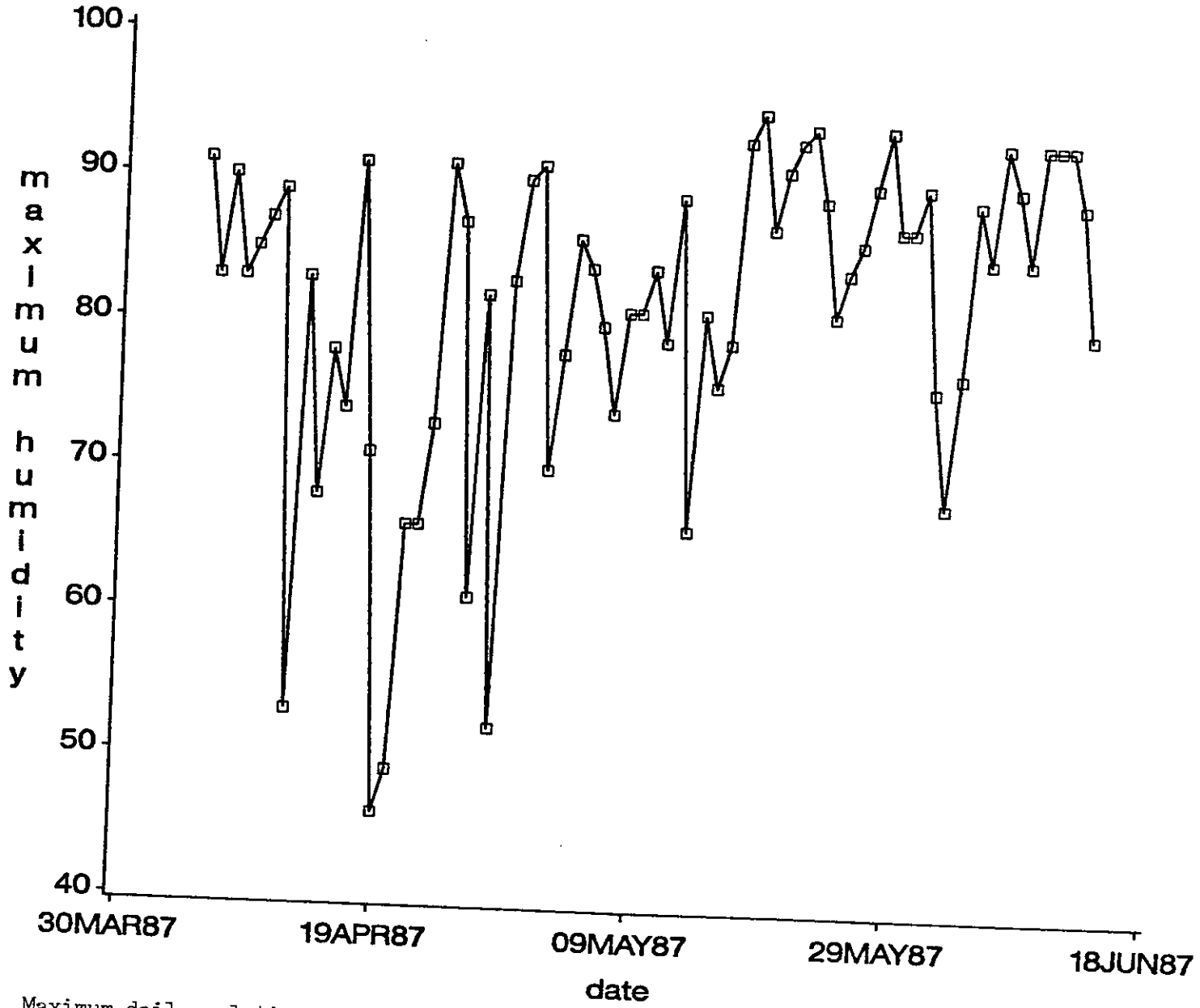
Maximum daily temperature (^{o}F) recorded in Brentwood, California, by the California Department of Water Resources, from 5 April, 1987 to 13 June, 1987. No data are available for 4-5 June.



Average daily relative humidity (x percent) recorded in Brentwood, California, by the California Department of Water Resources, from 5 April, 1987 to 13 June, 1987. No data are available for 4-5 June.



Minimum daily relative humidity (x percent) recorded in Brentwood, Califrnia, by the California Department of Water Resources, from 5 April, 1987 to 13 June, 1987. No data are available.



Maximum daily relative humidity (x percent) recorded in Brentwood, California, by the California Department of Water Resources, from 5 April, 1987 to 13 June, 1987. No data are available for 4-5 June.

Summary of meteorological data collected by EPA during the air sampling period one day after the first spray event.
Time: April 25, 1987: 12:15-12:45 pm

Parameter	Canopy	1M	1.5M	2M	3M	10M
Mean wind speed (m/sec)		1.4		1.4	1.4	1.9
Minimum wind speed (m/sec)		0.1		0.0	0.3	0.8
Maximum wind speed (m/sec)		2.9		2.8	2.8	3.5
Prevailing direction: (% of time)						
North		6		13	13	12
Northeast		0		0	0	0
East		0		0	0	0
Southeast		0		0	0	0
South		6		7	6	6
Southwest		38		20	32	25
West		25		41	25	42
Northwest		25		14	24	12
No measurable wind (% of time)		0		6	0	0
Mean temperature (C)	27.2	25.7		26.9	27.2	23.3
Minimum temperature (C)	25.9	24.9		25.9	25.9	21.9
Maximum temperature (C)	27.9	26.9		27.9	27.9	24.9
Relative humidity (%)		56				
Solar radiation (direct) (w/m2)			853			
Solar radiation (reflected) (w/m2)			66			
Mean vertical windspeed up (m/sec)		0				
% of time of upward readings		0				
Mean vertical windspeed down (m/sec)		0.1				
% of time downward readings		6				
% of time without vertical winds		94				

Summary of meteorological data collected by EPA during the background air sampling period one day prior to the second spray event.
Time: May 11, 1987: 12:51-1:21 pm

Parameter	Canopy	1M	1.5M	2M	3M	10M
Mean wind speed (m/sec)		1.3		1.5	1.3	1.5
Minimum wind speed (m/sec)		0.3		0.0	0.0	0.1
Maximum wind speed (m/sec)		2.6		2.9	2.9	2.9
Prevailing direction: (% of time)						
North		6		14	9	12
Northeast		6		0	0	0
East		0		0	0	0
Southeast		0		0	0	0
South		0		7	0	0
Southwest		37		21	27	24
West		31		35	36	42
Northwest		18		21	36	24
No measurable wind (% of time)		0		13	31	0
Mean temperature (C)	32.9	32.7		33.8	34.4	30.7
Minimum temperature (C)	31.9	30.9		32.9	33.9	29.9
Maximum temperature (C)	33.9	33.9		34.9	35.9	31.9
Relative humidity (%)		50				
Solar radiation (direct) (w/m2)			845			
Solar radiation (reflected) (w/m2)			56			
Mean vertical windspeed up (m/sec)		0				
% of time of upward readings		0				
Mean vertical windspeed down (m/sec)		0.1				
% of time downward readings		44				
% of time without vertical winds		56				

Summary of meteorological data collected by EPA during the second Frostban spray
Time: May 12, 1987: 8:27-8:42 and 10:03-10:18 pm

Parameter	Canopy	1M	1.5M	2M	3M	10M
Mean wind speed (m/sec)		0.6		0.8	0.2	1.7
Minimum wind speed (m/sec)		0.0		0.0	0.0	0.0
Maximum wind speed (m/sec)		2.1		2.4	2.6	3.8
Prevailing direction: (% of time)						
North		0		0	0	14
Northeast		70		53	50	54
East		16		39	50	34
Southeast		8		0	0	0
South		0		0	0	0
Southwest		0		0	0	0
West		8		8	0	0
Northwest		0		0	0	0
No measurable wind (% of time)		19		0	88	6
Mean temperature (C)	24.5	24.4		26.8	27.6	25.2
Minimum temperature (C)	22.9	22.9		24.9	25.9	23.9
Maximum temperature (C)	26.9	26.9		28.9	29.9	26.9
Relative humidity (%)			47			
Solar radiation (direct) (w/m2)			0			
Solar radiation (reflected) (w/m2)			0			
Mean vertical windspeed up (m/sec)		0				
% of time of upward readings		0				
Mean vertical windspeed down (m/sec)		0				
% of time downward readings		0				
% of time without vertical winds		100				

Summary of meteorological data collected by EPA during the air sampling period one day after the second spray event.
Time: May 13, 1987: 2:25-2:55 pm

Parameter	Canopy	1M	1.5M	2M	3M	10M
Mean wind speed (m/sec)		2.8		2.8	2.3	3.5
Minimum wind speed (m/sec)		1.2		1.3	0.5	1.9
Maximum wind speed (m/sec)		4.6		4.9	4.3	6.0
Prevailing direction: (% of time)						
North		18		36	30	6
Northeast		0		0	0	0
East		0		0	0	0
Southeast		0		0	0	0
South		0		0	0	0
Southwest		0		6	0	0
West		18		6	12	12
Northwest		65		53	60	83
No measurable wind (% of time)		0		0	0	0
Mean temperature (C)	33.7	32.8		33.3	33.9	29.9
Minimum temperature (C)	32.9	31.9		32.9	32.9	28.9
Maximum temperature (C)	33.9	32.9		33.9	33.9	30.9
Relative humidity (%)		39				
Solar radiation (direct) (w/m2)			518			
Solar radiation (reflected) (w/m2)			47			
Mean vertical windspeed up (m/sec)		0.1				
% of time of upward readings		6				
Mean vertical windspeed down (m/sec)		0				
% of time downward readings		0				
% of time without vertical winds		94				